J Food Sci Nutr.
Vol. 2, No. 3, p. 232~235(1997)

An Effects of Enzymatic Browning Reaction Products of Potato on the Antimutagenesis

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Abstract

This study was investigated to determine antimutagenic effects of enzymatic browning reaction products (PEBRPs) obtained by reaction of polyphenol compounds with oxidase extracted from potato. Catechol(Ca)-PEBRPs showed the strongest inhibitory effects with 90% inhibition on benzo-(α)-pyrene (B(α)P) induced mutagenesis in *Salmonella typhimurium* TA98, but the least with 40% inhibition on the 2-aminofluorene (2-AF) induced mutagenesis in TA98. The strong antimutagenic activities with 80% inhibition were observed in the presence of 100μg/plate of hydroquinone(HQ)-PEBRP on the B(α)P or 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole(Trp-P-1) induced mutagenesis in TA98, whereas HQ-PEBRP showed the least antimutagenic effect on 2-AF-induced mutagenesis. The addition of 100μg hydroxyhydroquinone(HHQ)-PEBRP to the plate led to approximately 82% inhibitory effects on 2-AF or Trp-P-1 induced mutagenesis in TA98, whereas the least antimutagenicity was observed in the 4-nitroquinoline-1-oxide(4-NQO) induced mutagenesis in the presence of 100μg/plate of HHQ-PEBRP. More than 80% inhibition were observed in the presence of 200μg/plate of Pyrogallol(Py)-PEBRP on the B(α)P or Trp-P-1 induced mutagenesis in TA98, but the least with 35% inhibition on 4-NQO induced mutagenesis in TA98. The results indicate that enzymatic browning reaction products of potato have a strong modulatory effect on mutagen induced mutagenesis in TA98.

Key words: enzymatic browning reaction products, antimutagenicity, potato

INTRODUCTION

Some substances in the natural product may have meaningful effects on the consequences of exposure to mutagens Recently, a study has been concerned about the cancer-preventing potential of naturally occurring constituents of the diets(1). Many workers have reported the antimutagenic effect of natural food components from plants, fruits or vegetables on the mutagenesis(2-4). These extracts are known to contain various antimutagenic substances which are capable of reducing the frequency of mutations induced with mutagens.

The Maillard reaction is considered to be one of the most predominant reactions occurring during the processing, storage, and preservation of some foods. Melanoidine, the main nonenzymatic browing products of the Maillard reaction, was demonstrated the inhibition of the mutagenesis induced by pyrolysates of amino acids and proteins during cooking(5). Also, the enzymatic browning reaction products of many fruits and vegetables such as apple, prune(yellow or red), and sweet potato have been reported to have antimutagenic properties(6-9). Thus, recently, it has been of great interest to investigate the physiological activity of enzymatically or nonenzymatically occurring browning reaction products. However, little works have been reported on antimutagenic effect of enzymatic browning reaction products of potato. In this study, inhibitory effects of enzymatic browning reaction products of potato(PEBRPs) on the 4-nitroquinoline-1-oxide(4-NQO), 2-aminoflurene(2-AF), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole(Trp-P-1), benzo(α)pyrene (B(α)P), and N-methyl-N'-nitro-N-nitrosoguanidine(MNNG) induced mutagenesis were observed.

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MATERIALS AND METHODS

Materials and Chemicals

The potato used was obtained from Pyungchang, Kangwon province in Korea. Pyrogallol(Py), hydroquinone(HQ), catechol(Ca), and hydroxyhydroquinone(HHQ) used as substrate for the browning reaction were from Kangwha company(Japan). Also, 4-NQO, B(α)P, 2-AF, Trp-P-1, and MNNG as mutagen were purchased from Sigma chemical company(USA).

Preparation of enzymatic browning reaction products of potato

Crude enzyme solution of potato was prepared by the Omura method(10) and stored at -20°C freezer prior to use. One gram of aceton powder obtained from above method was dissolved in 40ml of McIlvaine buffer(pH 6.0) and filtered with suction, then the solution was centrifuged at 10,000×g at 4°C for 30min and the supernatant was precipitated with ammonium sulfate. The sediment was dissolved in phosphate buffer(pH 6.1) and dialyzed with same buffer, then used as crude enzyme solution. Py, HHQ, HQ, and Ca were used as substrate for preparation of enzymatic browning reaction products of potato. The concentration of all substrates was 50mM and 100ml of the substrate solution was mixed with 5ml of crude enzyme solution and incubated at 30°C for 4 days with shaking, then the browning reaction product was dialyzed in the distilled water, and the dialyzed products was dehydrated. The browning reaction products were kept at -80°C and freeze dried prior to use.

Mutagenesis and antimutagenic activities of PEBRPs

The experiment was performed according to the preincubation method previously described(11), with or without B(α)P, 2-AF, Trp-P-1, 4-NQO, and MNNG. B(α)P, 2-AF, and Trp-P-1 required S9mix for antimutagenic test, but not for 4-NQO and MNNG. A mixed solution was prepared containing 0.25ml S9mix made from the rat liver S9 product(Organon Technica Corp. Durham, NC, USA), 0.1ml of cell suspension of *Salmonella typhimurium* TA98 and 0.1ml of each PEBRPs with each appropriate aliquot doses of B(α)P, 2-AF and Trp-P-1. But, for direct mutagen, such as MNNG and 4-NQO, a mixture solution was made without S9mix. The total volume of sample mixture was adjusted to 0.7ml with buffer. The prepared sample mixture was preincubated at 37°C for 20min, and then 2ml of molten top agar supplemented with L-histidine and D-biotin at 45°C was added to the mixtures, gently mixed and poured onto a minimal glucose agar plate. The plates were inverted and incubated at 37°C for 48h. Each set of experiments was performed at least twice and the results reported were obtained by counting the his+ revertant colony by using the following formula;

Inhibition ratio(%)=(A-C/A-B)×100

A : number of histidine revertant induced by a mutagen

B : number of revertants induced in the presence of PEBRPs alone and solvent(negative control)

C : number of histidine revertants induced by mutagen in the presence of PEBRPs

RESULTS AND DISCUSSION

The relationship between the doses of Ca-PEBRP and inhibition against the mutagenesis induced by 2-AF, B(α)P, 4-NQO, Trp-P-1, and MNNG are shown in Fig. 1. The Ca-PEBRP showed a dose-responding antimutagenic activity in *Salmonella typhimurium* TA98. Two hundred μg/plate of Ca-PEBRP led to the strongest antimutagenic acitivity with 90% inhibition on B(α)P induced mutagenesis in TA98 and 80% inhibition was observed in 2-AF, 4-NQO, and Trp-P-1 induced mutagenesis, but only 40% inhibition was occurred on the MNNG induced mutagenesis. The inhibitory effects of HQ-PEBRP on the mutagenesis induced by 2-AF, B(α)P, 4-NQO, Trp-P-1, and MNNG are shown in Fig. 2. Strong antimutagenic effects with 98% and 92% inhibition were observed in the presence of 150μg/plate of HQ-PEBRP on the B(α)P or Trp-P-1 induced mutagenesis in TA98, whereas HQ-PEBRP had antimutagenic activity with 18%, 25%, and 45% inhibition on MNNG, 4-NQO, and 2-AF induced mutagenesis, respectively.

Fig. 3 shows the inhibitory effects of HHQ-PEBRP on the mutagenesis induced by 2-AF, B(α)P, 4-NQO,

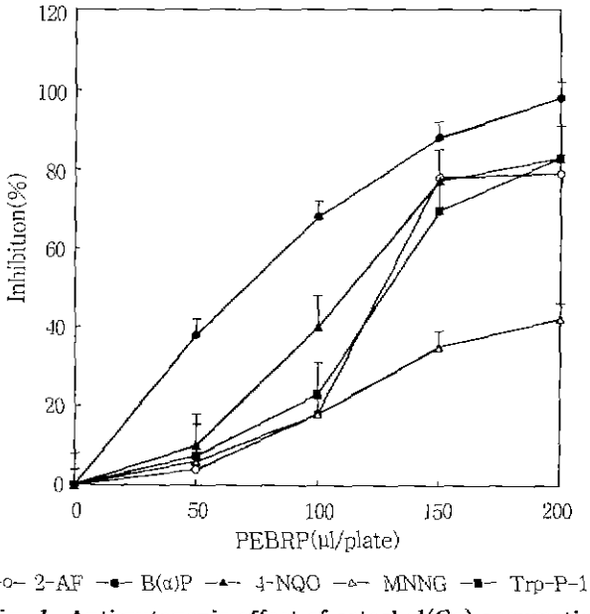

Fig. 1. Antimutagenic effect of catechol(Ca) enzymatic browning reaction product(PEBRP) on the designated mutagen induced mutagenesis in *Salmonella typhimurium* TA98.

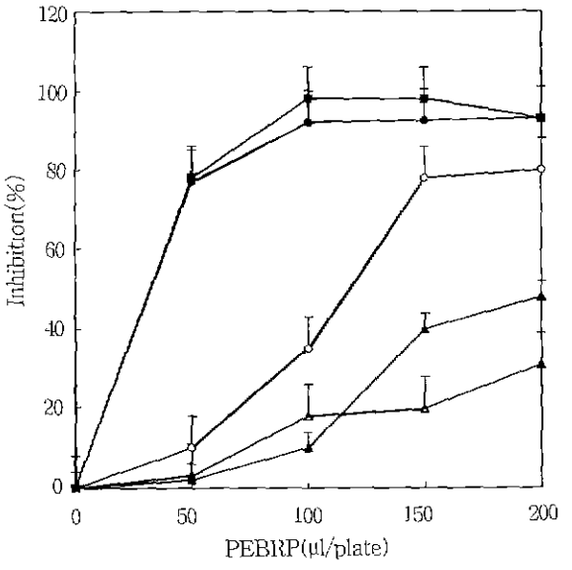

Fig. 2. Antimutagenic effect of hydroquinone(HQ) enzymatic browning reaction product(PEBRP) on the designated mutagen induced mutagenesis in *Salmonella typhimurium* TA98.

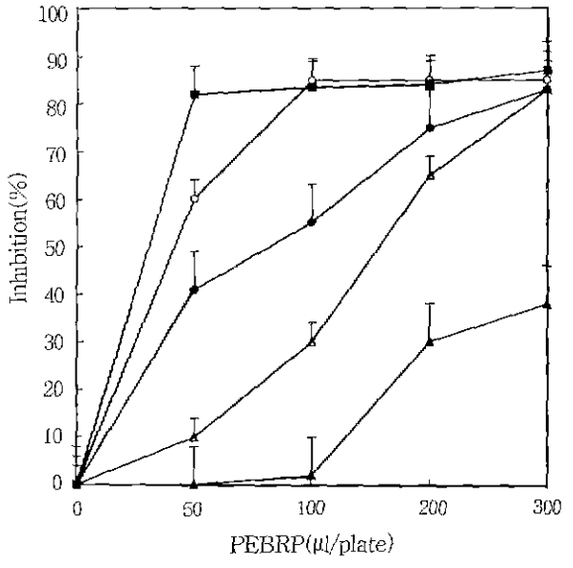

Fig. 3. Antimutagenic effect of hydroxyhydroquinone (HHQ) browning reaction product(PEBRP) on the designated mutagen induced mutagenesis in *Salmonella typhimurium* TA98.

Trp-P-1, and MNNG. The addition of 100μg PEBRP to the plate induced approximately 83% inhibitory effects on the mutagenesis induced by 2-AF and Trp-P-1 without further increase in higher concentration, whereas HHQ-PEBRP led to approximately antimutagenicity with 55%, 30%, and 20% inhibition on the B(α)P, MNNG, and 4-NQO induced mutagenesis in TA98 and antimutagenic effects of these three products increased in proportion to the concentration. The inhibitory effects of Py-PEBRP on the mutagenesis induced by 2-AF, B(α)P, 4-NQO, Trp-P-1, and MNNG are shown in Fig. 4. About 80% inhibition was observed in the presence of 200μg/plate of Py-PEBRP on the Trp-P-1 induced mutagenesis in TA 98 and followed by B(α)P, 2-AF, MNNG, and 4-NQO with 82%, 70%, 50%, and 35% inhibition, respectively. These results indicate that enzymatic browning reaction products of potato induce a regulatory effect on mutagen induced mutagenesis in TA98.

We investigated the effect of PEBRPs on the mutagenesis of TA98 before testing for the antimutagenicity of PEBRPs and the results showed that each PEBRPs itself did not influence upon their spontaneous mutation frequencies(data not shown). The present study show that enzymatic browning reaction product of potato have a regulatory effect on mutagen-induced mutagenesis. This is consistent with the previous results(6,7,9) indicating that enzymatic browning reaction products of fruits or

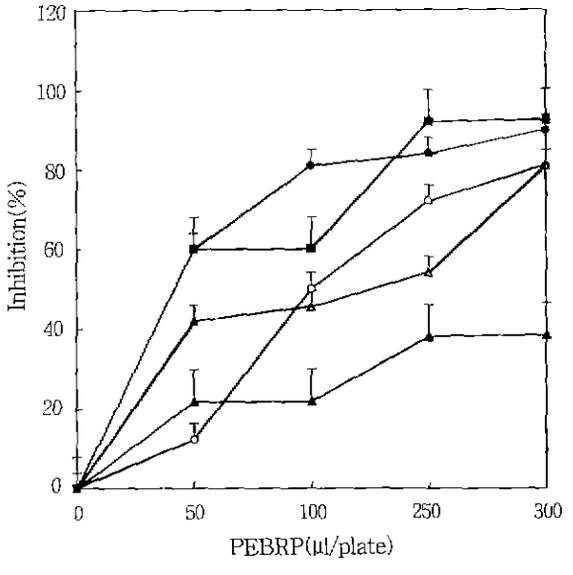

Fig. 4. Antimutagenic effect of pyrogallol(Py)-enzymatic browning reaction product(PEBRP) on the designated mutagen induced mutagenesis in *Salmonella typhimurium* TA98.

vegetables significantly inhibited the mutagenesis of several mutagens, such as MNNG, Trp-1, 2AF, or B(α)P in Ames test.

As plants like herbs, vegetables, or fruits contain various natural compounds, they may become a very valuable sources of the antimutagens and anticarcinogens in the future(12). The natural antimutagens or anticarcinogens

existing in our diets as a fresh or browning reaction products, especially fruits and vegetable are probably safe and these are inexpensive and easily available to obtain.

This study suggests that these browning reaction products may possibly play an important role as a versatile chemopreventive agent. Hence, it would be of interest to pursue these findings with more extensive studies, particularly on the identification of the main active substances in PEBRPs.

ACKNOWLEDGEMENTS

This work was supported by National Project Fund of the Misnistry of Education, Korea.

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(Received July 1, 1997)