Peroxidase Mediated Oxidation of Tyrosine: Tyrosine Hydroxylation in Tea Leaves

Peroxidase mediated oxidation of tyrosine to melanin has been demonstrated in the presence of dopa as cofactor, in mast cells, eosinophils, melanocytes, neurons and in horse radish[1-6]. However, PATEL et al.[7] have shown that plant and mammalian peroxidases oxidize tyrosine to melanin with either dopa or dihydroxy fumaric acid as cofactor and they have demonstrated that dopa and dopachrome are the intermediates in the process.

Materials and methods. Preparation of homogenate. Tea leaves, *Camellia sinensis* L. Kuntz were collected from Dehradun Valley. Homogenate was prepared by grinding tissue in Waring Blendor in water. The suspension was strained through 2 layers of muslin and made 10% (w/v).

Enzyme assay. The assay system for peroxidase activity was based on the method of PATEL et. al.[7] with some modifications. The final assay system in a total volume of 4.0 ml comprised: 2.0 mg L-tyrosine dissolved in 3.0 ml of 0.1 *M* acetate buffer (pH 5.5); 250 µg DL-dopa dissolved in 0.4 ml of the same buffer; 0.1 ml of 1.5% H_2O_2 and 704 µg ascorbic acid and 0.5 ml enzyme preparation. The reaction was carried out for 1 h at 25°C along with the control tube, without L-tyrosine, and it was stopped by the addition of 2.0 ml TCA (20%).

The dopa formed by the action of peroxidase was measured qualitatively and quantitatively. The specific colour reaction of dopa was carried out according to the method of ARNOW[8]. The difference in the intensity of color between experimental and control tube indicated dopa formation. It was further confirmed by descending paper chromotography using butanol: acetic acid: water (60:15:25, v/v) as solvent system. Spots were identified by ninhydrin spray. In quantitative estimation by spectrophotometric method[7,9,10], increase in absorbance at 281 nm between experimental and control tubes gave an indication of the amount of synthesis of dopa by tea-leaf perosidase system.

Results and discussion. It was noticed that enzyme, substrate concentrations and enzymic activity bear linear relationships for dopa synthesis. It was found that optimum substrate and enzyme concentrations were 2.0 mg and 0.5 ml of 10% homogenate respectively.

Peroxidase activity was assayed at pH 4.0, 4.5, 5.0, 5.5 employing 0.1 *M* acetate buffer, pH 6.0, 6.5 and 7.0 using phosphate buffer. In tea leaves, peroxidase activity was found to be optimum at pH 5.5 No reaction product was formed without enzyme or with boiled enzyme. No formation of dopa was noticed if H_2O_2 or dopa as cofactor was omitted.

It was noted in a preliminary experiment that ascorbic acid gave higher peroxidase activity if added before incubation than during homogenization. Therefore, other reducing agents used at their optimal concentrations, predetermined in every case, were added prior to incubation. The results showed that sodium bisulphite at 5×10^{-3} *M* increased peroxidase activity by 25% while cysteine, reduced glutathione and 2-mercaptoethanol and ascorbic acid at 1×10^{-3} *M* increased the activity to 35, 30, 40 and 300% respectively.

Qualitative and quantitative estimations revealed that dopa synthesis took place by tea leaf peroxidase system. The yield of dopa formed was found to be 1.2% calculated with respect to tyrosine conversion. The relevant data showing the effect of reducing agents on the formation of dopa are reported in the Table. It is evident from the Table that the formation of dopa in the presence of ascorbic acid was 4.8% as compared to 1.2% without ascorbic acid.

Dopachrome is formed from dopa in the absence of reducing agents because of the conversion of dopa to dopaquinone followed by further cyclization and finally it leads to the formation of melanin[10,11]. Dopaquinone is converted back to dopa in the presence of reducing agents especially ascorbic acid and exerts no interference with the hydroxylation of tyrosine[11].

Zusammenfassung. Untersuchungen über die Beeinflussung der Tyrosinhydroxylierung durch Peroxidase in Teeblättern (*Camellia sinensis* L.) mit Hilfe von Antooxydantien.

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Effect of reducing agents on the synthesis of dopa from tea leaf peroxidase mediated hydroxylation of tyrosine

Reducing agent	Dopa in µmole × 10^{-2}[a]	Yield of dopa (%)	Increase in the yield (%)
Nil	24.4	1.20	
Sodium bisulphite	30.5	1.50	25
Ascorbic acid	97.5	4.80	300
Cysteine hydrochloride	32.9	1.62	35
2-mercaptoethanol	31.7	1.56	30
Reduced glutathione	34.1	1.68	40

[a] Represents µmole of dopa formed from 1.0 ml of 10% (w/v) homogenate of tea leaves used under specified conditions of assay as described. Yield of dopa formation was calculated interms of tyrosine conversion to dopa. Supplements were used at their optimal concentrations. The concentrations were $5 \times 10^{-3}M$ for bisulphite and $1 \times 10^{-3}M$ for ascorbic acid, cysteine, 2-mercaptoethanol and reduced glutathione.

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