The Suppression of Catechol Oxidase Activity during the Enzymic Hydroxylation of *p*-Coumaric Acid by Spinach Leaf Phenolase

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The hydroxylation of *p*-coumaric acid to caffeic acid can be effected with a purified spinach leaf phenolase, which acts in the reaction as a mixed-function oxidase with a number of electron donors, notably ascorbate, NADH and 6,7-dimethyl-5,6,7,8-tetrahydropteridine. The enzyme also exhibits catechol oxidase activity (Vaughan & Butt, 1967, 1968). Molecular oxygen is utilized in both the hydroxylation of *p*-coumaric acid and the oxidation of caffeic acid to its *o*-quinone, which is reduced again by any of the reductants above. The further oxidation of caffeic acid is demonstrated by an oxygen consumption in excess of that required for its production.

When 10 μ moles of ascorbate was used in the hydroxylation of up to 10 μ moles of *p*-coumaric acid, oxygen uptake was linear with time but caffeic acid production, although initially equivalent to oxygen consumption, became slower. This decline was probably not due to an unfavourable competition between *p*-coumaric and caffeic acids, for the addition of 1 μ mole of caffeic acid, although increasing oxygen consumption by 80%, decreased only slightly the hydroxylation reaction.

The greater stability of catechol oxidase activity was further demonstrated with aged preparations, which formed caffeic acid more slowly without affecting the rate of oxygen consumption. The addition of bovine serum albumin restored the hydroxylation activity to that of the fresh preparations without altering oxygen uptake.

By contrast, with 2, 3 or 5 μ moles of dimethyltetrahydropteridine, oxygen consumption and caffeic acid production remained exactly equivalent throughout the reaction; the rates of each fell similarly as hydroxylation progressed. The addition of further quantities of the reductant restored the rates of caffeic acid production and oxygen consumption equally. A marked divergence between them was observed only when dimethyltetrahydropteridine was present in excess of *p*-coumaric acid.

With NADH, caffeic acid production fell below oxygen consumption as soon as hydroxylation commenced, even when non-catalytic protein was added. When dimethyltetrahydropteridine was added with NADH, however, catechol oxidase activity was completely suppressed.

It is suggested that hydroxylation requires some specific enzymic organization, which is unstable.

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Its maintenance during the reaction depends upon the nature of the electron donor and the relative concentration of *p*-coumaric acid. Catechol oxidase activity does not require the retention of this arrangement and survives the partial or complete loss of hydroxylase activity.

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An Alkaline Deoxyribonuclease from the Regenerating Liver of the Rat

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Alkaline deoxyribonucleases are found in a variety of mammalian tissues (Shugar & Sierakowska, 1967) but there is no evidence of structural identity between these enzymes (Laskowski, 1961). In rat liver the enzyme was shown to be associated with the mitochondria (Beaufay, Bendall, Baudhuin & de Duve, 1959) and proved to be a non-specific nuclease that degrades both RNA and denatured DNA (Curtis, Burdon & Smellie, 1966). A second alkaline deoxyribonuclease that preferentially hydrolyses native DNA can also be obtained from rat liver (O'Connor, 1968) and is described here.

The source of enzyme was the liver removed from Wistar rats 36 hr. after partial hepatectomy (Higgins & Anderson, 1931). The tissue was frozen on solid carbon dioxide, and then thawed and homogenized in potassium phosphate buffer, pH 8.0. From this homogenate the enzyme was purified at least 60-fold by a procedure that employed freeze-thawing, centrifugation at 105 000g, fractionation at pH 5, ammonium sulphate precipitation and chromatography on hydroxyapatite.

The purified enzyme was active over the range pH 7.0–8.0 but showed optimum activity in glycine-NaOH buffer, pH 8.0. In the presence of Mg^{2+} , activity was maximal at concentrations from 4–7 mM but decreased at higher concentrations. The cations Mn^{2+} , Zn^{2+} and Ca^{2+} all stimulated activity, but only to 50% of that shown with Mg^{2+} . For maximum activity the enzyme required a substrate with a high degree of secondary structure. It was three times more active with native DNA than with denatured DNA and was sensitive to the presence of partially denatured DNA. Although the preparation contained ribonuclease activity it was not associated with the deoxyribonuclease activity in