

*THE LOCALIZATION OF THE POLYPHENOL OXIDASE OF TEA LEAF

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Our interest in the polyphenol oxidase of tea was due to two reasons, firstly it is quantitatively the major enzyme of fresh tea leaf, and secondly, it plays an important part in the development, during black tea manufacture, of the quality and colour of tea liquors. Extensive studies have been made of this enzyme during the last 25-30 years, but only two of these have been concerned with determining the site of the enzyme in the tea leaf. The first of these was by Li & Bonner (1947), who proposed on the basis of differential centrifugation studies that polyphenol oxidase was associated with the chloroplasts. More recently, however, Takeo (1966) reported that the major part of the enzyme activity was not in the chloroplasts, but bound on smaller particles of the size of mitochondria.

In our study two experimental techniques were used one serological and the other chemical. In the former, transverse sections of tea leaf were stained with polyphenol oxidase antibody, which had been made fluorescent by conjugation with fluorescein isothiocyanate, followed by examination of the stained sections under a fluorescence microscope. The transverse sections of the leaf were cut on a freezing microtome, whilst the antigenic tea enzyme extract was prepared by grinding frozen tea "flush" (*ie* the terminal bud and two adjacent leaves), with sand and Polyclar AT, as described by Sanderson (1964). Examination of this extract by starch gel electrophoresis showed that four bands were present. These were detected by their fluorescence under ultra violet light, and by staining with Amido Black. All of them showed polyphenol oxidase activity on incubation with catechin or with di-hydroxy-phenylalanine, indicating that the protein present was this enzyme. Antibodies to this enzyme extract were engendered in rabbits by intravenous immunization, each rabbit being injected into the ear vein on five alternate days with increasing amounts of the tea leaf extract. The rabbits were test bled four to six days after the last injection, and the level of serum antibodies determined by a precipitin test. If the titre of antibody was above 1/1280, about 20 ml of blood were removed by cardiac puncture, and the serum conjugated in the cold (2°C) with fluorescein isothiocyanate. The conjugated protein was then separated from unreacted fluorescent material by passage through a Sephadex column, further purified by ammonium sulphate precipitation, and then used to stain the transverse sections of tea leaves. The staining techniques used were those described by Nairn (1964), and included direct, "sandwich" and "blocking" methods. On examination of the stained sections it was evident, as shown in slide 1 that, the polyphenol oxidase is localized in the epidermis and vascular bundles of the tea leaf. (Non-specific fluorescence, which appeared in leaf sections stained with conjugated normal rabbit serum, was shown by the sclereids and lignified xylem cells, as well as by the cuticle).

The identity of the enzyme as polyphenol oxidase was confirmed by making use of the fact that this enzyme oxidises epigallocatechin gallate to theaflavin gallate and other brown-coloured products. On treating transverse sections of young tea leaves with dilute solutions of epigallocatechin gallate, direct microscopic examination showed browning to have occurred in only the epidermis and vascular bundles. The presence of the polyphenol oxidase in the epidermis provides an explanation for the practical observation that "flaky" teas are associated with a "greenish" or raw taste of the liquors.

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Differences were however observed in the distribution of browning depending on the age of the leaf. Young leaves showed the presence of enzyme in both upper and lower epidermis, whilst in the third and older leaves, the enzyme was present in the lower epidermis only. These results were obtained by examination of leaves which had been stained with epigallocatechin gallate, as well as in leaves which had been caused to brown by treatment with chloroform vapour or by alternate freezing and thawing. These findings place in perspective the long-held assumption that the polyphenol oxidase of tea leaf is spatially separated from its substrates. Tambiah and co-workers (1966) have shown that the polyphenol substrates are located in the vacuoles of the palisade cells, and this spatial separation is evidently the reason why fresh, intact tea leaves do not turn brown, spontaneously, and also why maceration of the leaf is necessary for the initiation of fermentation during black tea manufacture.

Apart from its significance in the manufacture of black tea, it is possible that localization of the enzyme in the epidermis may confer protection against invasion by tea pathogens and pests, because rupture of the epidermis would enable the enzyme to reach and oxidise its substrates to theaflavins, quinonoid and other compounds which could combat the infection. Evidence in favour of this hypothesis is the observation that the Red Spider Mite *Oligonychus coffeae* Nietner shows a marked preference for older tea leaves and that it attacks these leaves only from the upper side (Fernando 1967) which is devoid of polyphenol oxidase. Another common pathogen of tea, *Exobasidium vexans* Masee which causes Blister Blight Leaf Disease, is intercellular in habit and takes care in avoiding rupture of the epidermis during the early post-penetration stages of growth (de Silva 1967).

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