Enzyme Histochemistry of Gum-resin Canals of Some Members of Burseraceae

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Activities of the enzymes peroxidase, acid phosphatase, succinate dehydrogenase, ATPase, amylase and lipase were localized in the epithelial cells of gum resin canals of Commiphora mukul and Boswellia serrata. The epithelial cells showed strong activity of peroxidase at various stages of gum-resin canal development. The activity of acid phosphatase was confined chiefly to the inner wall of epithelial cell facing the duct lumen. Higher levels of amylase as well as lipase were observed in the epithelial cells indicating the breakdown and conversion of starch and lipid into gum and resin components. Succinate dehydrogenase and ATPase show more intense reaction in epithelial cells than in the surrounding tissue. Possible role(s) of these enzymes in differentiation of the gum-resin canals as well as in the synthesis and secretion of gum-resin is discussed.

Key Words: Gum-resin canals, Commiphora mukul, Boswellia serrata, Enzyme histochemistry, Secretion

Introduction
Considerable information has accumulated during the past several years on the origin, development and distribution of gum, gum-resin and resin secretory canals in dicotyledenous and coniferous trees (Shah & Setia 1976, Venning 1948, Ghosh & Purkayastha 1960, Werker & Fahn 1969, Fahn 1979). Localization and distribution of different substances as well as ultrastructure of epithelial cells have been studied by several investigators (Fahn & Evert 1974, Fahn & Joel 1976, Setia et al. 1977). However, very little work has been done on enzyme distribution in the epithelial cells surrounding these canals (Stösser 1978). The present investigation was conducted to obtain some insight into the distributinal pattern of a few enzymes in gum-resin-yielding plants of family Burseraceae namely, Commiphora mukul and Boswellia serrata.

Materials and Methods
Young stems of Boswellia serrata and Commiphora mukul were collected from Dangs forest and ICAR Soil Conservation
Figures 1-6  
1-2. Peroxidase activity in epithelial cells of gum-resin canals in Commiphora mukul. Note the homogenous appearance of the enzyme in the epithelial cells in figure 2; 3-4 Boswellia serrata peroxidase activity in epithelial cells. Note granular appearance of enzyme; 5-6. Acid phosphatase activity in the epithelial cells. Enzyme is localized on tangential wall of epithelial cells. 5, C. mukul; 6, B. serrata
1 (x 80); 2 (x 700); 3 & 4 (x 400); 5 (x 700); 6 (x 1120).
Farms, Vasad (Gujarat) respectively. Thin fresh freehand sections were used to localize the enzymes. Histochemical localization of peroxidase, acid phosphatase, succinate dehydrogenase, ATPase and lipase were carried out following the schedules given by Bancroft (1975). The starch film method (Smith & Frommer 1973) was used to localize amylase activity. Photographs of histochemical preparations were taken with a Carl Zeiss Photomicroscope using Kodak photomicrography monochrome film SO-410.

Results and Discussion

The gum-resin canals in these members of family Burseraceae occur in most of the plant parts as reported earlier (Setia et al. 1977, Shah et al. unpublished). However, they are most abundant in the bark of the stem. The gum-resin canals are lined by a layer of epithelial cells, possibly the principal site of synthesis and secretion of the gum-resin. These cells are characterised by dense cytoplasm, conspicuous nuclei and high levels of nucleic acids, proteins, lipids and carbohydrates (Setia et al. 1977).

A strong peroxidase activity is observed in the epithelial cells of gum-resin canals of varying developmental stages in C. mukul (figures 1, 2) and B. serrata (figures 3, 4). In the epithelial cells of gum-resin canals in the secondary phloem of B. serrata, the activity appears in granular form (figure 4). The initials of canal as observed in C. mukul also show a very high peroxidase activity compared to surrounding cells. Association of intense peroxidase activity with the process of differentiation is well documented by Avers and Grimm (1959) in trichoblast and developing hairs, Mia and Pathak (1968) in sclereid and by Sethi and Malik (1974) in stomata. Peroxidase activity has also been reported in the areas of both lignification and cell division (Van Fleet 1959, Van Den Born 1963). However, the present investigation shows that besides differentiation, peroxidase plays some role in the synthesis and/or secretion of gum-resin. Our assumption is supported by the work of Heslop-Harrison (1972) and Poddubanaya-Arnoldi and Zinger (1961) who pointed out the association of peroxidase with secretory glandular organs.

The epithelial cells also exhibit a strong acid phosphatase activity. The activity which appears as black deposits is chiefly associated with the inner walls of the epithelial cells facing the duct lumen (figures 5, 6). The hydrolytic enzymes have some role in the dissolution of middle lamella of canal initials during the schizogenous development of the canals. Several electron micrographs show localization of acid phosphatase on cell walls as well as middle lamella region and separating zone of abscission tissue (Hall et al. 1977). According to Ghan and McLean (1968) acid phosphatase facilitates sugar transfer across the sieve plates by splitting the sugar phosphates. Pearse (1968) also reported role of phosphatases in intercellular transport. According to Onofeghara (1972) the exact role of phosphatases is unknown while Simola and Sopanen (1970) associated them with lipid synthesis.

Figures 7, 8 show intense reaction for succinate dehydrogenase in epithelial cells compared to the surrounding cells. The intracellular localization occurs as deep blue spherical or rod-shaped particles. It is an established fact that succinate dehydrogenase is a part of mitochondrial enzyme system and its strong activity in the epithelial cells indicates high mitochondrial or respiratory activity. The assumption is supported by the ultrastructural studies on C. mukul (Setia et al. 1977) and Pinus (Fahn & Benayoun 1976) which show the presence of numerous mitochondria in these
cells. In the studies on the enzyme distribution in the shoot tip of white spruce, Van Den Born (1963) observed intense succinate dehydrogenase activity in secretory cells of cortical resin canal. He attributed the activity mainly to the synthetic processes in the secretory cells. We believe that mitochondria through their enzyme system along with strong ATPase activity (figures 9, 10) play a vital role in supplying the energy required for the synthesis and transport of gum and resin. Our observations are in agreement with those of Stösser (1978) who also reported elevated activity of acid phosphatase, peroxidase and cytochrome oxidase during the course of gum duct formation in sweet cherries.

Amylase activity is very high in the epithelial cells (figure 12). It seems that starch grains are converted into soluble sugars or simple carbohydrates which in turn may be metabolized into gum-components. Similarly high activity of lipase in the epithelial cell (figure 11) is associated with breakdown of lipids into fatty acids which might be shunted into alternate pathways contributing to the formation of resin fraction. Brown et al. (1976) observed disappearance of starch and lipid, accompanied by increased synthesis of oleo-resin in slash pine after paraquat treatment.

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