LIPOXYGENASE IS ASSOCIATED WITH OIL BODY MEMBRANES IN MATURE OLIVE ENDOSPERMS

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Lipoxygenases (LOX) are enzymes that use molecular oxygen to produce hydroperoxides from fatty acids that have a *cis,cis*-pentadiene structure. A LOX form has been identified as an oil body membrane protein in cucumber and soybean cotyledons (1), and in anise seedlings (2) Oil bodies from olive seed endosperm and embryo tissues contain about 10% (w/w) protein and oleosins have been immunodetected (3). Recently we found that LOX activity is present in olive oil extracts (4). In this work LOX enzymatic activity has been detected in high molecular mass fractions of olive endosperm extracts and LOX is proposed to be an oil body membrane protein in olives.

Mature olives (*Olea europaea* cv. microcarpa or mastoids) koroneiki variety, were generously gifted by the National Agricultural Research Foundation, Subtropics and Olive Institute of Chania, Greece

Oil bodies of olive endosperms were isolated by ultracentrifugation and LOX activity of their suspension was compared with that of the soluble supernatant; LOX content was found to be higher in the soluble fraction. This fraction was further chromatographed on a Sepharose 4B column and the LOX catalytic activity was found to be almost entirely associated with high Mr components. Light microscopy revealed that these fractions contain oil bodies, suggesting that LOX is an oil body membrane enzyme in olive endosperms SDS-polyacrylamide gel electrophoresis of delipidated proteins present in high Mr fractions revealed the presence of a 100 kDa band, possibly corresponding to the LOX enzyme Protein bands of lower molecular mass were also observed, apparently corresponding to oleosins, the oil body structural proteins.

Total phosphate content was measured in LOX active fractions and calculated to be about 12 nmol mg^{-1} of dry mass, suggesting the presence of phospholipids (PL). Neutral lipids of the LOX active fractions were extracted with diethyl ether, weighed and calculated to be about 0 4 mg per ml of active fraction. Proteins and PL were separated by chloroform methanol (2 1, v/v) extraction The chloroform fraction containing PL was chromatographed on a TLC plate (silica gel 60A) and the presence of *á*-phosphatidylcholine was revealed

Nordihydroguaiaretic acid (0 3 mM), a known LOX inhibitor, completely inhibited LOX activity Heat treatment of the active fractions revealed heat sensitivity of the enzyme In contrast, LOX was very stable at low temperatures. Optimum pH was found to be 6 0, while the activity was remarkably reduced when pH was higher than 6.5. The substrate dependence was examined using both linoleic and linolenic acid as substrates. K_m was calculated to be 0 20 0 03 and 0 25 0 04 mM respectively. Aggregation and coalescence of lipid bodies were studied by a turbidimetric procedure (5). According to this method the isoelectric point of oil bodies was calculated to be about 6.0.

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