INACTIVATION OF A FUNGAL LACCASE BY FREE PHENOXYL RADICALS

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Introduction. Laccases (LAC) are oxidoreductases that contain Cu ions capable of catalyzing the oxidation of phenols, following a free radical mechanism (1). Phenols are toxic substances of environmental concern. Hence, the application of LAC has been considered for the remediation of polluted water and soil (2). However, in other oxidoreductases, such as peroxidases, the free phenoxyl radicals react with the active site of the enzyme, leading to activity loss (3). The rate of enzyme inactivation could be related to the redox potential (*E*) of the phenol in two ways: high *E* phenols would be more inactivating, whereas phenols with lower *E* are better substrates for the enzyme, thus producing more radicals. The objective of this work is to study the inactivation process of a fungal LAC by free phenoxyl radicals with variable *E*.

Methods. LAC from *Coriolopsis gallica* UAMH 8260 was purified from a crude extract as described in (4). Inactivation profiles were obtained by incubating the enzyme with variable concentrations of phenol (Ph) (*E*=0.97 V) and 4-methoxyphenol (4MPh) (*E*=0.72 V) and measuring activity decay as the reaction progresses with a syringaldazine colorimetric assay.

Results. Inactivation profiles show that the rate of enzyme activity loss depends on initial substrate concentration [Si] (Fig. 1, 2). For the same substrate and as expected, inactivation occurs faster at higher [Si]. As for different substrates, we observed that at the same [Si] (1 mM) and after a 10 min reaction, LAC residual activity was aprox. 26% for 4MPh, whereas for Ph was aprox. 85%. It is possible that, because 4MPh is a better substrate than Ph, the concentration of free radicals is higher and thus the inactivation proceeds faster. It is interesting to notice that at low 4MPh concentrations, the inactivation of LAC is not complete. We also observed high amounts of insoluble polymer formed in the reaction with 4MPh, in agreement with a higher concentration of free radicals. However, total protein measurements showed that almost all the protein is associated with the polymer.

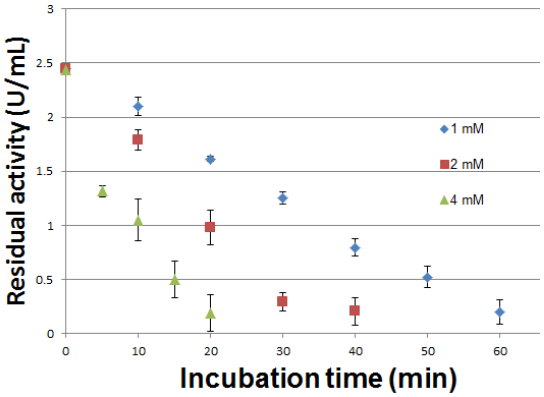

Fig. 1. Inactivation of LAC in the reaction with Ph.

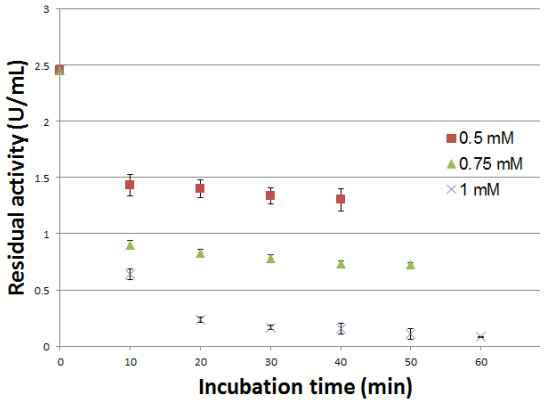

Fig. 2. Inactivation of LAC in the reaction with 4MPh.

Conclusions. Our data suggest that the redox potential of the substrate strongly relates to the inactivation rate of the enzyme. The polymer formed in the reaction could have a previously unidentified role in the inactivation process.

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