

Detection of Free Radicals Formation in Haemolymph of Insects by EPR Spectroscopy

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Abstract. The generation of reactive oxygen species during oxidation of DOPA (3-(3,4-dihydroxyphenyl)-DL-alanine) in haemolymph of insects has been studied by electron paramagnetic resonance with spin traps 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide and 1-hydroxy-3-carboxy-pyrroline. The data support the formation of DOPA-derived highly reactive intermediates during melanization in haemolymph of insects. The formation of superoxide has not been detected apparently due to reaction of DOPA with superoxide. The rate constant of the latter reaction was estimated as $k \sim 5 \cdot 10^5 \text{ M}^{-1}\text{s}^{-1}$. The formation of DOPA-semiquinone in haemolymph has been measured using spin stabilization of *o*-semiquinones by Mg^{2+} . The comparative studies in haemolymph of intact insects and insects infected by fungal infection suggest an important role of DOPA-semiquinone production in the immune status of insects.

1 Introduction

The immune system of invertebrates comprises a variety of mechanisms and elements of defense against all types of pathogens or parasites. Melanotic encapsulation and the production of cytotoxic reactive intermediates are the important components of this defense. A multilayer cover containing different quinones and melanins is formed around pathogens during the encapsulation process. The main role in melanization belongs to enzyme phenoloxidase (PO), which converts mono- and/or diphenols (in particular, DOPA (3-(3,4-dihydroxyphenyl)-DL-alanine)) to quinones with their following polymerization to melanins [1, 2]. During this process the highly reactive quinoid intermediates can be generated, including DOPA-semiquinone [3, 4]. They can be involved in cytotoxic reactions in defense mechanism of insects [5, 6] and/or can interact with oxygen reducing it to a superoxide anion [7, 8]. The latter might result in the formation of other