Abstract

The cytochromes P450 are among the most important enzymes involved in the biotransformation of xenobiotics in the body. They are part of the moooxygenase system that interact with other enzymes - NADPH:cytochrome P450 reductase and cytochrome b5. Mutual interaction of enzymes in mooxygenase system are not completely solved. Covalent crosslinking technique could contribute to clarify the possible protein-protein interactions and their consequences. One of the possible realization of this plan is to use photoactivatable cytochrome P450, which after exposure to UV radiation created covalent complex with components of monooxygenase system, with which it is in contact. Therefore, this paper focuses on developing optimal conditions for the production of recombinant cytochrome P450 2B4 in order to gain knowledge for the production of photoactivatable cytochrome P450 with incorporated amino acids L-photomethionine and L-photoleucine. In experiments was cytochrome P450 2B4 expressed in two strains of *Escherichia coli*, C41 (DE3) and BL21 (DE3) Gold, and two culture flasks, glass Erlenmeyer flask and plastic Fernbach flask. During expression optical density of the bacterial suspension (absorbation at 600 nm) and concentration of cytochrome P450 were measured. Methodology of measuring the concentration of cytochrome P450 in the bacterial cell has been developed. Expressed cytochrome P450 was solubilized and subsequently found its specific content in solubilizated solution. Optimal conditions for production of recombinant cytochrome P450 2B4 have been proposed on the basis of comparison of the bacterial expression in different cultivation arrangements.

Keywords:
expression of the protein, cytochrome P450, photoactivation, crosslinking, MFO system