

Abstract

The cytochrome P450 system plays an important role in metabolism of endogenous compounds and xenobiotics. This system consists of cytochrome P450, NADPH:cytochrome P450 oxidoreductase (CPR), cytochrome b₅ and NADH:cytochrome b₅ reductase (CYB5R3). Explanation of protein-protein interactions among these reaction partners is essential for understanding the function of the entire system. Covalent cross-linking is a favorable method for studying these interactions. In this work a photo-activatable analogue of amino acid L-methionine (L-photo-methionine) was used as a cross-linking agent.

This work is focused on the organic synthesis of L-photo-methionine, expression and isolation of CPR and CYB5R3 as photoactivable proteins containing incorporated L-photo-methionine. Auxotrophic strain of *E.coli* B834 (DE3) and minimal media were used for the expression. CYB5R3 with incorporated L-photo-methionine was successfully expressed and isolated. The extent of L-photo-methionine incorporation was verified by mass spectrometry. Furthermore, the photo-initiated cross-linking of CYB5R3 with cytochrome b₅ was tested.

Key words: photolabile amino acid, protein expression, synthesis