Abstract

Mixed-function oxidases play a major role in the metabolism of xenobiotics. The main component of this system is the cytochrome P450, it oxidizes substrates coming into our body to more polar products. Another component of mixed-function system – the cytochrome b₅ (cyt b₅) is able to modulate the function of cytochrome P450, the mechanism of this modulation is yet unknown. However, it is believed that it could be mediated via transfer of electron or allosteric modulation of cytochrome P450 caused by interaction with cyt b₅. The aim of this thesis was to find and prepare analogs of cyt b₅, which are unable to transfer electrons to cytochrome P450 and simultaneously are structurally very similar to native cyt b₅. The conformational stability of cyt b₅ and its analogs was monitored using pulse proteolysis. This method employs proteases to cleave the evaluated protein at varying concentration of a denaturant. For soluble proteins, urea is typically used as denaturant in combination with thermolysin as protease. While for membrane proteins, sodium dodecyl sulfate (SDS) is usually used as denaturant together with subtilisin as protease. The aim of this thesis was to use these methods to compare a conformational stability of the native human cyt b₅ with apo-cyt b₅ and analogs of the cyt b₅ reconstituted with Mn³⁺ protoporphyrin IX (Mn³⁺ PPIX), Cr³⁺ protoporphyrin IX (Cr³⁺ PPIX) and Co³⁺ protoporphyrin IX (Co³⁺ PPIX). Reconstitution and preparation of cyt b₅ containing hemin or its analogs via titration and gel chromatography is also described.

Key words: electrophoresis, pulse proteolysis, hemoproteins.

(In Czech)