

Abstract

The cytochromes P450 (P450s) are important enzymes involved in metabolic pathways, which use exogenous and endogenous substances as their substrate for various enzymatic reactions. These enzymes can also use precarcinogens as their substrate and activate them into carcinogens, which leads to a cancer development. If the P450s are induced, the cancer risk increases. Some chemopreventive compounds may induce the P450s and thus be harmful to the human body. Therefore it is necessary to pay enough attention to a study of the mechanism of action of P450s and the influence of the chemopreventive compounds on the activity of cytochromes P450.

mRNA expression of most of the P450s isoforms is detected in a number of healthy (nontransformed) tissues, *viz.* liver, brain, heart, colon, kidney or placenta. Nevertheless there are a few P450s isoforms which mRNAs are expressed at relatively low levels in the nontransformed tissues, whereas the expression in the transformed tissues is significantly higher. One of these P450s is CYP2W1, which can be used as a prognostic marker for colorectal cancer - therefore it is useful to be able to detect a presence of this enzyme in various tissues. A detection of P450s can be accomplished by using a method Western blot. In this method, the immunodetection is achieved by using specific antibodies. The mammalian antibodies (IgG) are for this purpose used the most but also the antibodies isolated from egg yolk (IgY) are getting popular in the last years.

In this diploma thesis, two peptide sequences were chosen from the primary structure of CYP2W1 and conjugated to a keyhole limpet hemocyanin (KLH). After the immunization of hens by these immunogens the IgY were isolated by precipitation method with sodium chloride. The next step was purification by affinity chromatography, which led to obtain specific antibodies. At the end, the purified IgY were used for the immunodetection of CYP2W1 in cell lysates and their efficacy was compared to the efficacy of antibodies produced in rabbit.