UNIVERZITA KARLOVA V PRAZE FARMACEUTICKÁ FAKULTA V HRADCI KRÁLOVÉ

Téma diplomové práce	**Studies of carbonyl reductases 1 and 3 variants with emphasis on S-nitrosoglutathione as substrate and inactivator**
Jméno studenta, studentky	**Tereza Hartmanová**
Jméno oponenta	**prof. RNDr. Eva Kvasničková, CSc.**

II. Posudek oponenta

The topic of the thesis is in line with the interests of both laboratories (Faculty of Medicine, Christian-Albrechts University in Kiel and Faculty of Pharmacy, Charles University in Prague), which have studied the properties, catalytic activity, structure, and substrate specificity of human type carbonyl reductases CBR1 and CBR3. Though the two enzymes are highly similar at the amino acid level (display 72% sequence homology), they are currently known to differ in their substrate specificities and play different metabolic roles. The author has undertaken a challenging project with the aim to identify the amino acids in the sequence of CBR1 responsible for its catalytic properties, since the enzyme has broader substrate specificity. The goal was to suitably modify the CBR3 AA sequence and create several variants - certain number of AA residues of these enzyme were replaced to correspond to CBR1.

The paper is very well prepared and written, presented data are supported by self-explanatory figures and tables; the list of references is comprehensive. Both the topic and the scope of the thesis go well beyond the framework and requirements of standard graduate thesis.

Questions and topics for discussion:

1. Why do you consider S-nitrosoglutathion to be so important a substrate for the measurement of the activity of the two enzymes
2. What widely used drugs in clinical medicine are metabolized (deactivated or activated) by the enzymes
3. Your methodology covers a wide spectrum of methods inclusive a whole array of molecular biology, biochemical and chemical techniques. Could you briefly identify the most difficult methodological problem you had to tackle when working on your research project?
4. Your results with the CBR3 mutant are splendid and the finding that its activity is inhibited by higher concentrations of the substrate (GSNO) is interesting – do you have an explanation for this effect?
5. I thing that it would be very interesting to further study the reactivation of CBR1 activity as you plan.

In my opinion this is a high quality diploma work. The experimental part of the project was time intensive, required considerable knowledge and laboratory skills and, last but not least, a lot of patience.

I recommend the thesis for defense and propose Excellent

Navrhovaná klasifikace **excelent**

V Hradci Králové dne

Podpis oponenta diplomové práce