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Peer review on the PhD thesis of Hillary Elizabeth Hoffman

CHARACTERIZATION OF RECOMBINANT HUMAN SERINE RACEMASE

Range of work: 65 pgs divided into Summary, List of Abbreviations, Introduction, Results, Discussion, References and Appendix (Reprints of author's publications described in the thesis)

Aims:

There were three major aims of this thesis. The first was to establish a system for recombinant expression and purification of human serine racemase (SR) and to compare recombinant human and mouse SR in terms of their kinetic parameters and inhibitor sensitivity. The second aim was to identify potent specific SR inhibitors for use in tissue culture and animal model experiments, where aliphatic hydroxamic acids as a novel class of SR inhibitors were introduced. Last, but not least aim of the presented thesis was the experimental determination of 3D structure of human SR. The method for generation and screening of random human SR mutants contributing to elucidation of the SR structure-functional relationships within the enzyme was described.

Main results:

1. Active recombinant human serine racemase was produced in *E. coli* and the purified enzyme was kinetically characterized using an HPLC-based assay. Like the mouse ortholog, human SR (hSR) exhibited both racemization and elimination activity and showed very similar kinetic parameters (K_m and k_{cat}) to those found for mouse ortholog. Also exhibited inhibitor–sensitivity represented by K_i values was similar within both orthologs. The obtained results validate the use of mouse models for study of SR inhibition.

2. The screening experiments concerning SR inhibitors were performed to identify novel structures capable of SR inhibition. As effective inhibitor hydroxamic acids were

disclosed and their mechanism of action was investigated. In addition, the mechanism of potent hydroxamic acid SR competitive inhibitor (L-aspartic acid β-hydroxamate) which modified the pyridoxal-5′-phosphate cofactor was characterized in details.

3. To solve the 3D SR structure the random mutagenesis of hSR was carried out. Although the numerous SR constructs were screened, no diffracting crystals were obtained. Despite the lack of an SR crystal some information about structurally and functionally important residues as key for enzymatic activity were clarified.

Reviewer comments:

1) In paper 1 you used for the determination of mechanism of inhibition and Ki the reaction mixture preincubated with inhibitor for 15 minutes before addition of L-serine. Do you think that in this case you can speak about competitive inhibition?
2) Could you explain in more details the role of Mg^{2+} and ATP in activation of your enzyme? You expect the chelatation of Mg^{2+} in inhibition reactions with hydroxamic acids. Do you take into account the chelatation of Mg^{2+} in complex with ATP?
3) D-amino acids are often found in toxic proteins. Do you know if there are some proteases in eukaryotic organisms which are able to hydrolyze these proteins?
4) Do you know if there are some hypotheses concerning prevalent occurrence of L-form of amino acids in living organisms?

Conclusion:

This work meets demands set to PhD thesis, because author proved her ability to work independently, to draw original conclusions from experimental data obtained by advanced scientific methods, and to summarize all in papers that were accepted in renowned international scientific journal. The thesis is written clearly and without errors, the proofs are clear and easily understandable. I think that the results will be surely of considerable interest to specialists working in the field.

Prague, 26.1. 2010

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