

Poster Presentation

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The trans-membrane 17 β HSD7: Kinetic study and preliminary crystallization data

J. Theriault¹, D. Zhu¹, S. Lin¹

¹Centre de recherche du CHUL (CHU de Quebec), Departement de medecine moleculaire, Quebec, Canada

Human 17 β -Hydroxysteroid dehydrogenase type 7 (h17HSD7) is a transmembrane enzyme belonging to the large, phylogenetically related protein family of short-chain dehydrogenases/reductases (SDR). In this study, h17HSD7 was expressed, purified, and the steady-state kinetics was studied. Here we report a purification method of the transmembrane enzyme using FPLC equipment with a combination of detergents to obtain homogeneous protein. We also report steady-state kinetics for h17HSD7 at 37 degrees Celsius and pH 7.5, using a homogeneous enzyme preparation with estrone (E1), or dihydrotestosterone (DHT) as substrate and NADPH as the cofactor. Kinetic studies made over a wide range of concentrations of both steroids (2microM to 60microM) revealed that E1 (Km= 12microM) has a higher apparent affinity for the h17HSD7 compared to DHT (Km= 44microM). Crystallization of h17HSD7 in complex with high concentration of steroids to saturate the enzyme in the drop as low apparent affinity of both steroids was found in kinetic study. Some preliminary crystals were obtained by hanging-drop and diffracted at synchrotron facility (Maximum resolution: 2.95Å, Space group: H 3 2, Average unit cell: 120.74 120.74 201.94 90 90 90, Mosaicity : 0.51).

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