Inhibition of human α-L-fucosidase

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ABSTRACT

A gene from human encoding α-L-fucosidase (AFU) was cloned into pET22b plasmid. Protein was successfully expressed in *E. coli* BL21 (DE3). After applying a series of ion-exchange and gel-filtration chromatography purification steps, recombinant AFU with 95% homogeneity can be obtained. The molecular weight of the enzyme was analyzed by SDS-PAGE to be about 50 kDa.

pH-dependent study indicated that AFU exhibited 2 optimal regions at pH 4.5 and pH 6.5, and the enzyme would become unstable when pH is lower than 3.5 or higher than 7.5. Michaelis constant (K_m) and catalytic activity were determined with *p*-nitrophenyl-α-L-fucopyranoside (PNPF) and were found to be 0.105 mM and 48.6 sec^{-1}, respective. Comparing with AFU extracted from human liver (K_m = 0.43 mM and maximal velocity = 19.6 μmole/mg/min equal to k_{cat} = 16.3 sec^{-1})，the catalytic power (k_{cat}/K_m) of recombinant AFU is 12-fold stronger than native human liver AFU.

In order to investigate the reaction mechanism of AFU, a series of aryl-α-L-fucopyranoside were synthesized for Brønsted relationship study. The Brønsted constant β_{lg} is -0.27 obtained from Brønsted plot constructed with a series of aryl-α-L-fucopyranoside with p*K*a > 7.0.

Initial burst also observed during the enzyme reaction. Based on these two preliminary results, the catalytic mechanism of AFU was purposed to be a two-step double displacement mechanism with the rate-limiting step at the deglucosylation step.

Inhibitor of AFU was also screened. A chemical library from Spectrum Collection® （MicroSource）was used as drug candidate for screening. Fortunately, several inhibitors were found to be effective such as irreversible inhibitor: Cisplatin, Ebselen; competitive inhibitors: Ethambutol、Mitoxantrone and uncompetitive inhibitor: Dequalinium chloride. Cisplatin and Ebselen would covalently bond to the amino acid residue cysteine of AFU; hence, the active site structure of the enzyme may be changed and finally lost its activity through this bonding. The K_i value of Ethambutol for human AFU was 23 μM； specific inhibition study for different glycoside hydrolases and AFU from different sources with Ethambutol was also investigated. Ethambutol is also one of the first-line medications for pulmonary tuberculosis, was found to be a specific inhibitor of recombinant human AFU.