EVALUATION AND EFFECTIVENESS OF ENZYMATIC OXALATE REDUCTION IN THE PREVENTION OF STONE FORMATION

Hypothesis: Routine monitoring will enable patients to become aware of oxalate levels and to take measurements on a daily basis to reduce possible recurrence of kidney stones or other oxalate-related diseases through changes in diet and fluid intake.

Project Objective: To develop a dipstick test to measure urinary oxalate levels.

METHODS

A codon optimized synthetic OxOx gene derived from Hordeum vulgare (barley) was generated and cloned into the EcoRI/Xbal sites of the pPICZαA expression vector downstream of the N-terminal alpha mating factor secretion peptide sequence and used for expression in Pichia pastoris X-33 strain. This expression system is well suited for our purpose since it is relatively rapid, inexpensive, and capable of generating disulfide bonds and addition of glycans required for the functional expression of this enzyme. Another major advantage of using this secretion system is that it functions as a first step in the process of protein purification. Since the pl of this OxOx enzyme is predicted to be 5.5, anion exchange chromatography was used for purification (Q-sepharose: equilibrium/binding with tris buffer pH 9.0 and elution with 1M NaCl). Kinetic assays were performed at optimal enzyme conditions as noted in the figure legend.

RESULTS

A. OxOx Plasmid Construction

ml

B. Plasmid DNA Sequencing

C. Nucleotide Blast Alignment

RESULTS SUMMARY

We identified an active oxalate sequence derived from barley and cloned a synthetic gene into the yeast expression plasmid pPICZαA. P. pastoris was transformed with the OxOx expression plasmid and induced to express active enzyme. Culture media was subsequently dialyzed overnight against distilled water. Active enzyme was purified to greater than 90% purity using Q-sepharose anion exchange chromatography. Our purified OxOx enzyme displayed standard Michaelis Menten kinetics at substrate concentrations up to 400µM (after which a well-known substrate inhibition occurs) and had an estimated Km value of 256µM based on linear regression analysis using a Lineweaver-Burk plot.

CONCLUSIONS

- Generated an active Oxalate Oxidase yeast secretion expression system
- Purified OxOx enzyme using anion exchange chromatography
- Determined OxOx enzyme kinetics
- Future Direction: We plan to optimize the expression and purification protocols for this enzyme while developing a first-generation oxalate dipstick prototype.

ACKNOWLEDGEMENTS

This work was supported by the Urology Care Foundation Research Scholar Award Program, The Endourological Society, The University of Florida Department of Urology, and the National Institutes of Health Grant 5T32DK009769-05.

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EXPRESSION OF BARLEY OXALATE OXIDASE ENZYME USING A PICHIA PASTORIS SECRETION SYSTEM

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INTRODUCTION

- Roughly 80% of kidney stones contain calcium oxalate
- Urinary oxalate is a key factor in estimating kidney stone risk
- Part of the 24-hour urine stone profile
- There is no simple and quick point-of-care oxalate test for clinicians and patients


RESULTS

A. OxOx Expression Time-course

B. Anion Exchange Purification

C. Post-purification Coomassie Staining

LC-MS/MS Analysis

Fig. 2. OxOx Expression and Purification. A. P. pastoris culture media samples were taken every 24 hours to evaluate expression by western blotting using anti-OxOx antibodies. B. Culture media was dialyzed overnight against distilled water. The pH was adjusted to 9.0 using a tri buffer, applied to a Q-sepharose strong anion exchange column, and purified OxOx enzyme was eluted with 1M NaCl. OxOx expression was verified by anion exchange chromatography and total protein was visualized with Coomassie stain to determine purity.

LC-MS/MS analysis of total protein from the OxOx expression and purification process was conducted on a Q1000 mass spectrometer equipped with a nano-ACQUITY UPLC system. The protein samples were analyzed using a reversed-phase gradient with a C18 phase that eluted at a flow rate of 600 µl/min. The system was calibrated using a high-purity water standard. OxOx expression and purification was performed in 3 separate batches. The avg. yield of OxOx from the final batch was 15.6 mg/L as determined by SDS-PAGE analysis.

CONCLUSIONS

- Generated an active Oxalate Oxidase yeast secretion expression system
- Purified OxOx enzyme using anion exchange chromatography
- Determined OxOx enzyme kinetics
- Future Direction: We plan to optimize the expression and purification protocols for this enzyme while developing a first-generation oxalate dipstick prototype.