

Cellulase (E1) characterization

Goal:

Determination of Km (Michaelis-Menten constant) and Kcat (catalytic constant) of the enzyme, as a free as well as a surface-displayed enzyme, using a fluorescent substrate: 4-methylumbelliflferone. We expect an increase in fluorescence in with E1 cellulase concentrations, up to the point where the substrate concentration becomes a limiting factor.

Tested cellulase

E1 endo-1,4- β -D-glucanase (from *Acidothermus cellulolyticus*).

General protocol

Repeat a set of ascending E1 cellulase concentrations with reaction buffer containing 4 - methylumbelliferyl β -D-celllobioside (MUC) in excess. Terminate the reaction with stop mix (pH 10) and determine fluorescence with a fluorescence microtiter plate reader.

Later, repeat a set of ascending 4-MUC concentrations with a fixed cellulase concentration

Controls

- Control A – negative control – contains only reaction buffer (without cellulase). We expect no reaction, and we want to verify there is no spontaneous reaction of our substrate during the experiment period.
- Control B contains cellulase (without reaction buffer). We expect no reaction since there is no substrate.

Detailed protocol

Part 1 - optimal cellulose concentration:

1. Use a 96-well microtiter plate (flat-bottomed, polystyrene plate, black).
2. Prepare the reaction buffer containing:
 - a. 50 mM sodium acetate pH 5.5
 - b. 100 mM NaCl
 - c. 0.5 mM 4- methylumbelliferyl β -D-celllobioside (MUC)
3. Load 100 μ l reaction buffer in each well.
4. Load 0.1, 0.5, 1, 2, 3, 4, 10 units of the cellulase to be assayed.
5. Prepare control wells:
 - a. Control A: contains only 100 μ l reaction buffer.
 - b. Control B: Contains 10 units of assayed cellulase + adequate amount of DDW (Double Distilled Water) to reach 100 μ l.
6. Cover plates with adhesive lids to prevent evaporation and incubate for 30 min at 65 °C.
7. Terminate the reaction by adding 100 μ l of stop mix (0.15 M glycine pH 10.0).
8. determine fluorescence with a fluorescence microtiter plate reader using excitation and emission wavelengths of 365 nm and 455 nm, respectively.

9. Compare fluorescence values to control A (blank, contains reaction buffer).

Part 2 - optimal 4-MUC concentration:

10. Prepare reaction buffer with different 4-MUC concentrations: 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 5 mM 4-methylumbelliferyl β -D-celllobioside (MUC).
11. Repeat the experiment with the optimal cellulose concentration found in Part 1.

Resources

1. T. Ziegelhoffer, J.A. Raasch, S. Austin-Phillips, **Dramatic effects of truncation and sub-cellular targeting on the accumulation of recombinant microbial cellulase in tobacco**, Mol. Breed., 8 (2001), pp. 147-158
<https://link.springer.com/content/pdf/10.1023/A:1013338312948.pdf>
2. Lehmann, C., Sibilla, F., Maugeri, Z., Streit, W. R., de María, P. D., Martinez, R., & Schwaneberg, U. (2012). **Reengineering CelA2 cellulase for hydrolysis in aqueous solutions of deep eutectic solvents and concentrated seawater**. Green Chemistry, 14(10), 2719-2726.
https://pubs.rsc.org/en/content/articlehtml/2012/gc/c2gc35790a?casa_tok_en=bXySI7kkm7kAAAAA:eaU2GRcouW9JIV7jCc7sBNPWCfVBqUzlsdu-x8nrl9OZ92HA0kDGVZbFJZ_U-qOiXJtHxhP2e_Nt3QZU