Cellulose Inhibition on Alpha-Amylase Enzyme and Possible Health Application via a Cellulose-Rich Diet

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Forewords—*It is highly recommended viewing this paper in PDF format on a media device where possible because of the color-coded tables. Or Appendix section to be printed and read in color.*

REALAbstract—Diabetes type II is a life-threatening and life-detrimental disease that is possible to acquire, as it is a consequence of how we live out our life, what kind of food we consume because a wise man once said (on multiple occasions): "We are what we eat." Our experiment tried to find out whether or not a modification in our daily intake of nutrition could result in offsetting the onset of type II diabetes. We suspected that cellulose might be the inhibitor of glucose synthesis – the main contributor to type II diabetes – by "fooling" amylase with its similar molecular structure. We tested our hypothesis using 15-minute fixed-time assay with varying concentrations of cellulose at room temperature.

1. INTRODUCTION

Type II diabetes mellitus is a health problem denoted by high blood sugar level (hyperglycemia), particularly glucose. This is caused by the body's being inert to insulin as a result of being exposed to high insulin concentration on a lengthy basis and rendering the cell's unable to absorb glucose from blood. Type II diabetes is both detrimental to the patient's life quality as well as their financial situation. Thus, preventing or at the very least defer the onset of type II diabetes is absolutely critical to a healthy and satisfying life.

To achieve this, we can seek to decrease the glucose amount present in blood and effectively lower the amount of insulin our body needs. We know that the enzyme alpha-amylase is the main contributor in increasing glucose in bloodstream as this enzyme hydrolyzes polysaccharides, i.e., starch and glycogen, to make glucose and maltose. Through enzyme inhibition by a naturally-occurring inhibitor that can be conveniently added to our diet, we can hopefully slow down the speed at which glucose is made in the body. Even though our body mainly needs cellulose for the digestion happens in the intestinal tract in the form of dry mass (or fibre), cellulose can perhaps be an inhibitor to alpha-amylase. Since a cellulose monomer has a closely resembling structure (called beta-1,4 D-glucose) to that of starch and glycogen, it can slow down glucose production by *tricking* alpha-amylase into binding cellulose on its active site.

There is a high possibility that cellulose would help to inhibit the alpha-amylase hydrolysis process, even though the exact mechanism is up for further study. This experiment is designed to verify such hypothesis and to see if adding more cellulose in one's diet therapeutically push back the onset of type II diabetes and ultimately improve one's life quality.

2. MATERIALS

The material for this experiment is provided by the college biology laboratory. These materials are necessary to avoid replication crisis for this experiment.

- Sterile Eppendorf tubes to transport substances
- Microcrystalline cellulose (3.33 mg/L)

- Amylose (2 mg/mL) in 0.1 M phosphate buffer pH 7
- Maltose (2 mg/mL)
- Phosphate buffer at pH 7
- Alpha-amylase (0.02 mg/L) in 0.1 M phosphate buffer at pH 7
- 0.1 M HCl
- Iodine reagent (5 mM I_2/5 mM KCl)
- 96-well plates
- Micropipettes and tips
- Timer

3. PROCEDURES

3.1. Standard curve preparation

- Start the dilutions by adding these amounts of amylose (2 mg/mL) and phosphate acetate buffer into 6 test tubes. Refer to the following **Table 1**.

Table 1. Dilution steps of Amylose for the creation of standard curve using iodine reagent and spectrophotometry

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Amylose $4\frac{g}{L}$ (μL)	**0**	**50**	**100**	**150**	**200**	**250**
0.1 M phosphate buffer (μL)	**500**	**450**	**400**	**350**	**300**	**250**
[Amylose] mg/L	**0**	**0.4**	**0.8**	**1.2**	**1.6**	**2.0**

- Label 3 sets of 6 wells on the 96-well plate for trial 1, 2, and 3. Then **dispense $40\mu L$** of phosphate buffer into each of the labeled 18 wells.
- Dispense $40\mu L$ of amylose solution from each of the 6 tubes into each of the 6 wells in trial 1, respectively.
- Repeat the step above for trial 2 and 3 wells.
- Incubate the current at room temperature for 10 min.
- Add $20\mu L$ HCl 0.1 M into all 18 wells.
- Reserved until after all samples other than the standard curve have been prepared and incubated: Dispense $100\mu L$ of iodine reagent to ALL the samples.

3.2. Measuring alpha-amylase activity in the presence of alleged inhibitor cellulose

- Dilute the stock microcrystalline cellulose solution with the phosphate buffer solution as shown in the following **Table 2** in the *Appendix*. **All of which are put into 8 Eppendorf tubes labeled in accordance.**
- These next steps utilize the wells according to Table 3 shown in **Table 3** in the *Appendix* and the calculated concentrations from **Table 2**.
- **IMPORTANT:** dispensing substances in Table 3 must follow a set time interval between each dispensation. Most likely **30 seconds** due to time restraints.
- A**fterwards,** once every well has been prepared, now it is safe to add the iodine in every sample and put the plate in the spectrophotometer for readings.

3.3. Some notes:

- **The order of dispensation:** Enzyme alpha-amylase → [inhibitor] → amylose (timed interval) → iodine reagent.

4. Results

5. Discussion

Appendix

Table 2. Dilution steps of Cellulose test substance in a buffered environment

Tube	1	2	3	4	5	6	7	8
V_{buffer} (μL)	400	300	250	200	150	100	50	0
$V_{cellulose}$ (μL)	0	100	150	200	250	300	350	400
$C_{cellulose}$ (mg/mL)	0	0.625	0.9375	1.25	1.5625	1.875	2.1875	2.5

Table 3. Conditioning Cellulose in different environments and concentrations

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
No AO or AA	NC	50 *B*	50 *B*	50 *B*	50 *B*	50 *B*	50 *B*	50 *B*
AO	AO	C2, 40 B + 10 AO	C3, 40 B + 10 AO	C4, 40 B + 10 AO	C5, 40 B + 10 AO	C6, 40 B + 10 AO	C7, 40 B + 10 AO	C8, 40 B + 10 AO
AO + AA	AO + AA	C2, 10 AO + 10 AA	C3, 10 AO + 10 AA	C4, 10 AO + 10 AA	1C5, 0 AO + 10 AA	C6, 10 AO + 10 AA	C7, 10 AO + 10 AA	C8, 10 AO + 10 AA

Legend: AO = amylose, AA = amylase, NC = no compound, B = phosphate buffer, volume units succeeding all numerical values = μL . E.g. C6, $20\ B + 10\ AO\ 0.2 + 10\ AA\ = [Inhibitor]_6 + 20\ \mu L\ buffer + 10\ \mu L\ Amylose\ 0.2\ mg/L + 10\ \mu L\ Amylase$

Table 4. 96-well plate detailed layout

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	SC 1: 40 B + 40 C1	SC 2: 40 B + 40 C2	SC 3: 40 B + 40 C3	SC 4: 40 B + 40 C4	SC 5: 40 B + 40 C5	SC 6: 40 B + 40 C6	SC 1: 40 B + 40 C1	SC 2: 40 B + 40 C2	SC 3: 40 B + 40 C3	SC 4: 40 B + 40 C4	SC 5: 40 B + 40 C5	SC 6: 40 B + 40 C6
B	SC 1: 40 B + 40 C1	SC 2: 40 B + 40 C2	SC 3: 40 B + 40 C3	SC 4: 40 B + 40 C4	SC 5: 40 B + 40 C5	SC 6: 40 B + 40 C6						
C	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO
D	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO
E	50 B + 30 C1	50 B + 30 C2	50 B + 30 C3	50 B + 30 C4	50 B + 30 C5	50 B + 30 C6	50 B + 30 C7	50 B + 30 C8	50 B + 30 C1	50 B + 30 C2	50 B + 30 C3	50 B + 30 C4
F	50 B + 30 C5	50 B + 30 C6	50 B + 30 C7	50 B + 30 C8	50 B + 30 C1	50 B + 30 C2	50 B + 30 C3	50 B + 30 C4	50 B + 30 C5	50 B + 30 C6	50 B + 30 C7	50 B + 30 C8
G	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO
H	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO	40 B + 30 C1 + 10 AO	40 B + 30 C2 + 10 AO	40 B + 30 C3 + 10 AO	40 B + 30 C4 + 10 AO	40 B + 30 C5 + 10 AO	40 B + 30 C6 + 10 AO	40 B + 30 C7 + 10 AO	40 B + 30 C8 + 10 AO

Legend: SC = standard curve, B = phosphate buffer, C = concentration code according to Table 2, AA = amylase, AO = amylose. All units succeeding volumetric values are in μL. Red, Green, Blue denote trial 1, 2, 3 respectively.

Table 5. Absorbance value of experimentally collected samples at 450nm wavelength

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.055	0.317	0.599	1.444	1.177	1.667	0.049	0.322	0.641	0.948	2.249	2.617
B	0.064	0.514	1.487	1.388	1.897	1.562						
C	0.697	0.776	0.708	1.049	0.906	1.265	0.787	0.983	0.693	0.758	0.737	0.943
D	0.84	1.031	0.809	1.255	0.745	0.76	0.933	0.822	0.879	0.886	0.938	1.209
E	0.333	0.722	0.316	0.338	0.301	0.302	0.312	0.277	0.303	0.297	0.317	0.296
F	0.293	0.294	0.303	0.496	0.281	0.305	0.286	0.298	0.305	0.299	0.302	0.395
G	0.807	0.699	0.749	0.724	0.877	0.794	0.659	0.984	0.957	0.722	0.89	0.872
H	0.851	0.797	0.702	0.803	0.8	0.741	0.787	0.824	0.826	0.89	0.894	1.032

Legend: Red, Green, Blue denote trial 1, 2, 3 respectively. Purple denotes empty wells.