Enzymes: Salivary Amylase

HEREDITY & HUMAN AFFAIRS

SPRING 2012

LAB #6

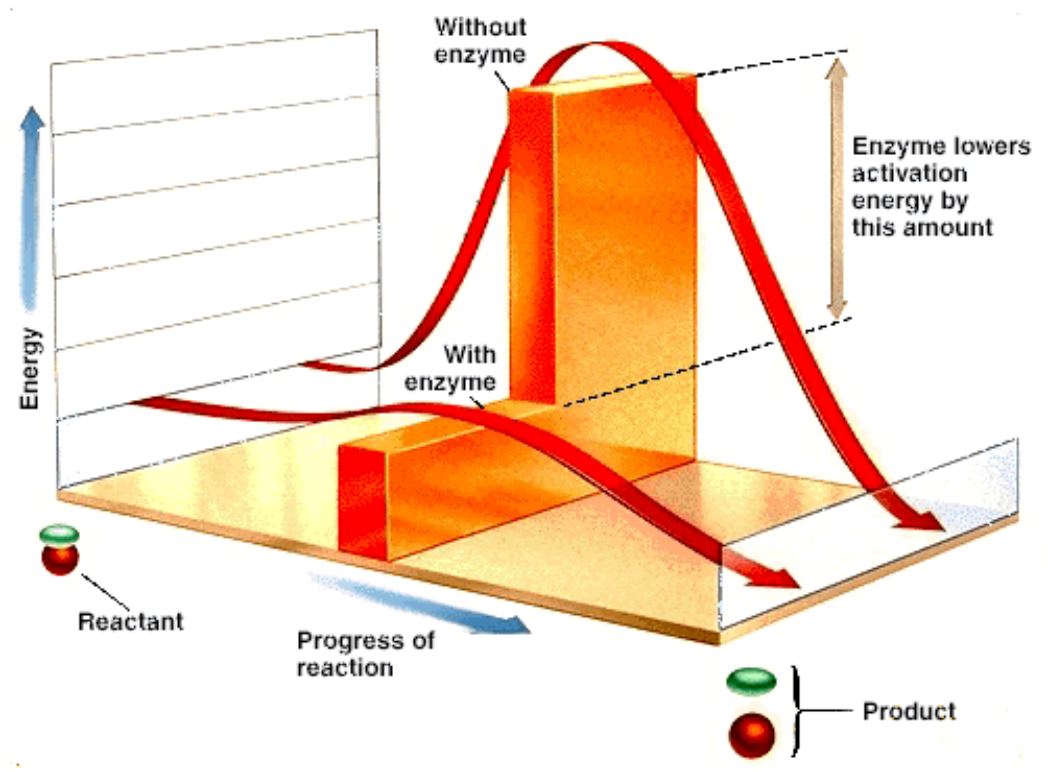
What are Enzymes?

- Enzymes comprise largest & most diverse group of **PROTEINS**.

- Act as **biological catalysts** → cause or accelerate chemical reactions by lowering the Energy of Activation (E_a)

Biological Catalysts

- Note the reduction in energy necessary to complete reaction when enzyme is present.
- Sort of like conserving energy!



Properties of Enzymes:

- **Large protein** molecules
- **Re-usable**
  - e.g. H_2O_2 *(catalase)*→ $2\ H_2O + 1\ O_2$
- Remain **unchanged**
- **Very specific** – act only on specific substrate
  - "Lock & Key Fit"
- Operate at **very high speeds**
  - e.g. catalase can break down 2 million H_2O_2 per minute at 0°C!
- **Rate** of reaction is dependent upon **temperature, pH, [E], & [S]**
- *Usually* work best below 60°C
- **Denature** at very high temperatures (e.g. 100°C)

Naming Enzymes:

- **Trivial**
  - e.g. trypsin & pepsin
- **Substrate + "ase" ending**
  - e.g. maltase
- **Action + "ase" ending**
  - e.g. oxidase (adds oxygen); dehydrogenase (removes H^+ from substrate)
- **Combo + "ase" ending**
  - e.g. succinic acid dehydrogenase (substrate = succinic acid, action = removes H+, enzyme = ase ending)
- **Numerical**
  - e.g. used more often in chemistry

Genetics & enzymes

- Recall:

  DNA → RNA → Proteins

- Proteins, therefore Enzymes, have a genetic basis!

Salivary Amylase

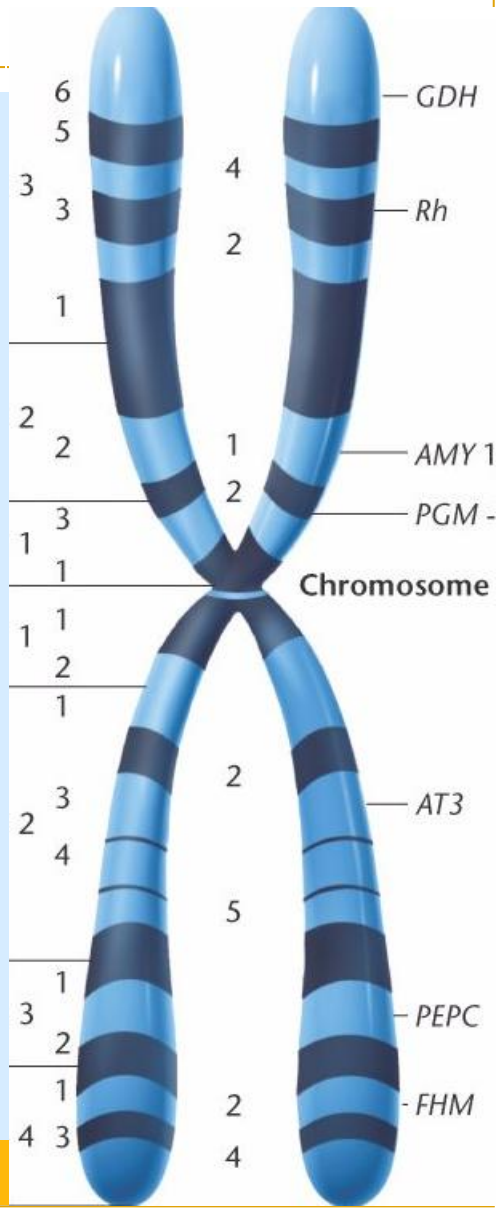
- Salivary Amylase- Digestive enzyme responsible for catalyzing the reaction:

Starch → Maltose

- Example: you put a cracker in your mouth and let it stay there (no chewing!). What happens?
  - Salivary Amylase in your Saliva begins to digest (break down) the starch!

Salivary Amylase - genetics

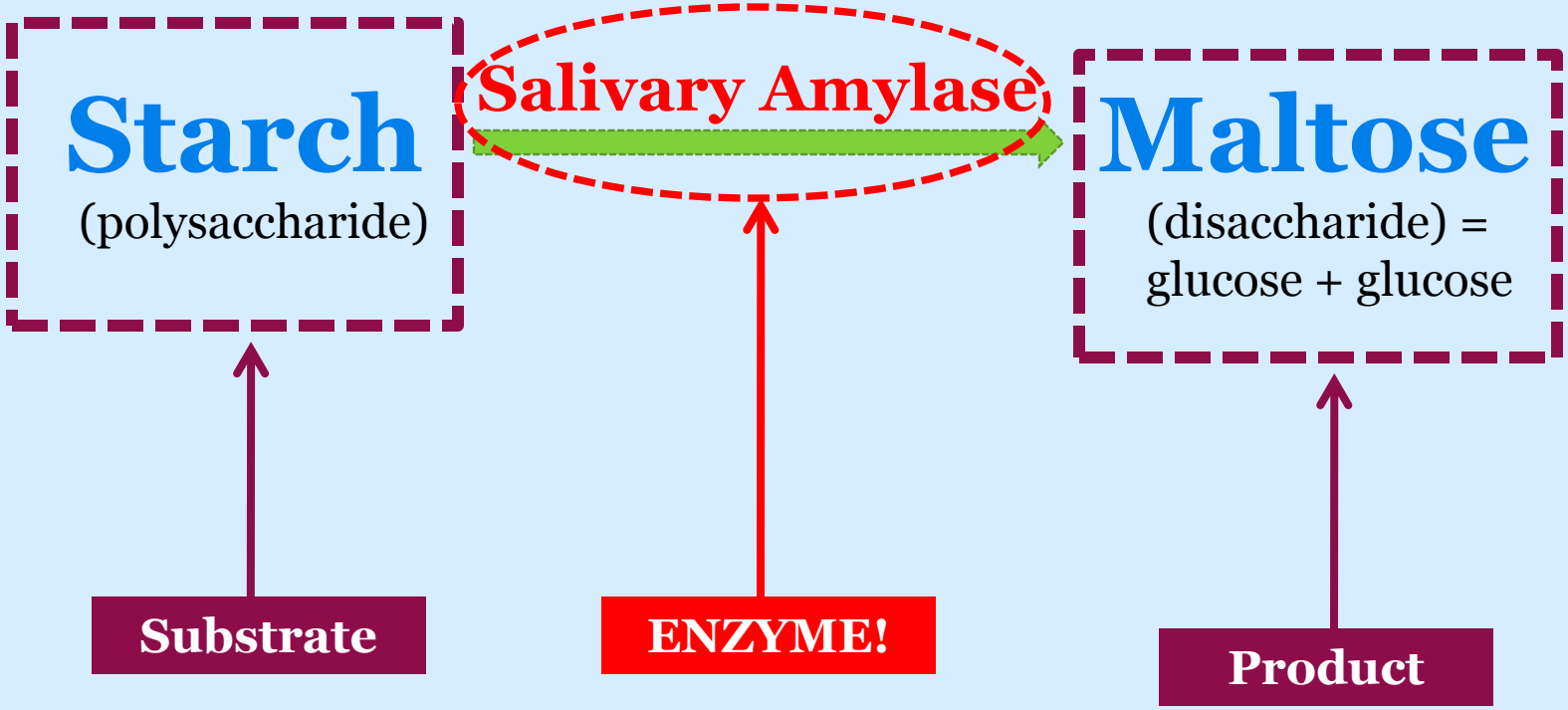
- Gene found on Chromosome #1
  - Location: 1p21
- Genes AMY1A, AMY1B, AMY2C
  - 3 isoforms (i.e. different forms of the protein)
- # of gene copies correlates with level of salivary amylase produced
- # of gene copies associated to evolutionary exposure to high-starch diets



The Basic Reaction

Substrate $\xrightarrow{\text{enzyme}}$ **Product**

Today's Reaction

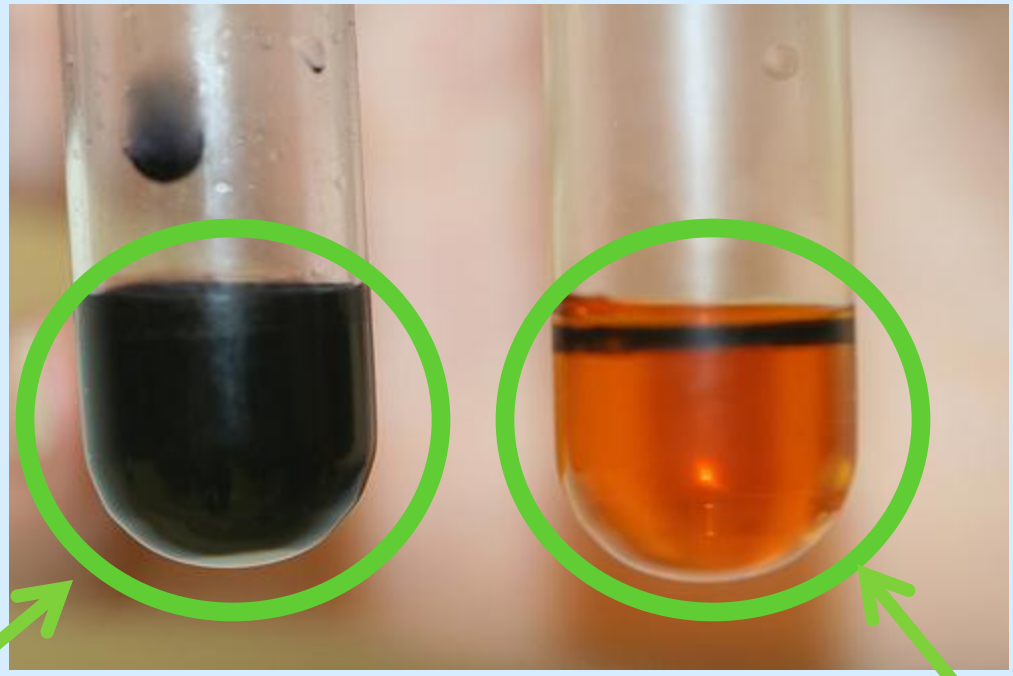


Today's Reaction

Starch $\xrightarrow{\text{Salivary Amylase}}$ **Maltose**

- In today's experiment, you will monitor the breakdown of starch into maltose.
- The speed of the reaction (reaction rate) will be dependent upon the **temperature**.
  - The reaction rate will also partially depend on your salivary amylase genes.
- Upon adding of Lugol's Iodine (starch indicator), watch for solution to turn from purple/black to amber.

Starch $\xrightarrow{\text{Salivary Amylase}}$ Maltose



Purple/black = lots of starch present!

Amber/yellow =no starch present

CAUTION!

- Saliva should only be handled by the person from which it originated!
- Everyone must wear **<u>gloves</u>** & **<u>goggles</u>** when working with saliva.
- All glass test plates & test tubes must be **disinfected** with **<u>10% bleach</u>**, then washed with soap & water.
  - Keep the glass plates.
  - Dispose of the used test tubes in glass disposal box (front of the lab).

Fun Facts About Saliva!

- In a day….

  You will produce 1.7 liters of saliva

- In a lifetime….

  You will produce about 10,000 gallons

Fun Facts About Saliva!

- Your mouth c n microbes!
  - Mostly bacteri
- A kiss can con cteria, 95% of which
  - May have evol
  - Help build resi

Procedure Overview:

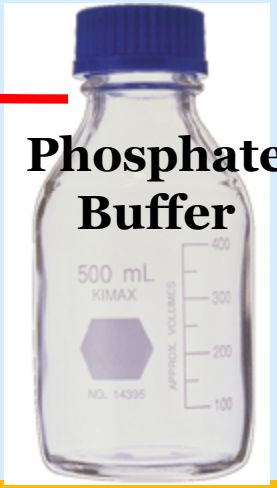
1. Deposit saliva in Dixi-cup (one person/pair).

Spit into this cup!
You need at least 1.5 ml liquid saliva
(not foam/bubbles)

2. Prepare "Saliva/Buffer Mixture" (1:1 ratio)

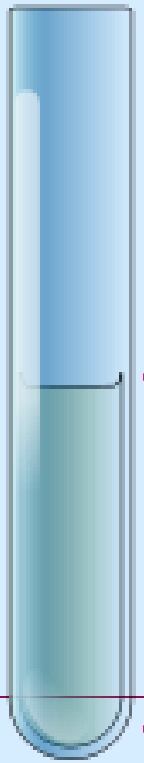
Add 1.5 ml

Add 1.5 ml



Procedure Overview:

3. Saliva-Buffer Mixture. Mix well & Label this tube.
Never add anything to this tube;Only remove.
This is your enzyme for all experiments!!!

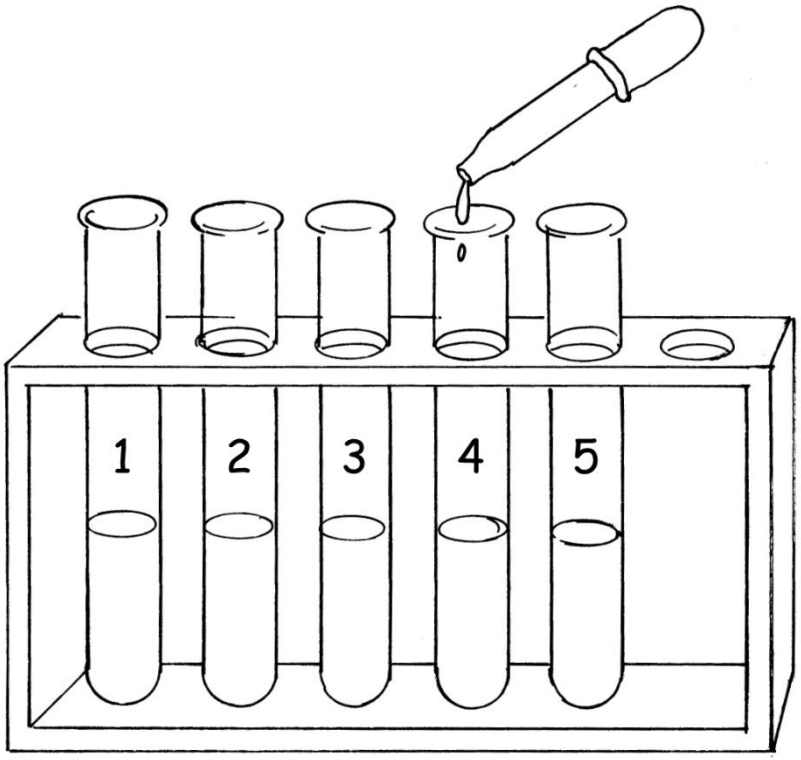


1.5 ml saliva +
1.5 ml buffer =
3 ml "Stock Saliva/Buffer"

Procedure Overview:

4. Fill 5 test tubes with 5 ml each Starch Solution. (These are your substrate tubes).
   - Label with **initials** & experimental **temperatures**.
   - Put into respective water baths & allow to come to temp.

What do you expect to be the ***optimal temperature*** for Salivary Amylase?
(In other words, at which temperature does this enzyme work best? Why?



Label:
0°C
20°C
40°C
60°C
80°C

Procedure Overview:

5. Start with Room Temperature (20°C).
6. You must work **quickly** with your lab partner.
   - 1 person uses timer & adds Lugol's Iodine.
   - 1 person adds "reaction mixture" (starch with saliva-buffer drops).

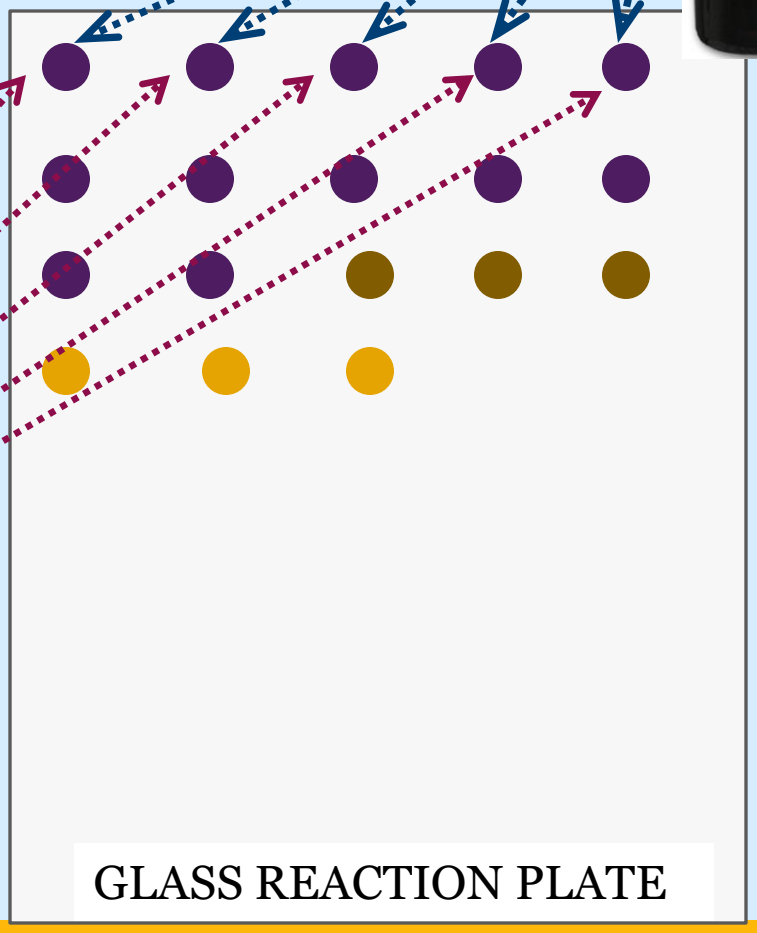
IMPORTANT: Once reaction begins, you must immediately start timing!

Procedure Overview:

Stock: Saliva/Buffer (~3 ml total)

Add **5 drops** Saliva/Buffer Mix to the Starch tube & mix well. Reaction starts **NOW**!!!

Starch @ 20°C (5 ml)



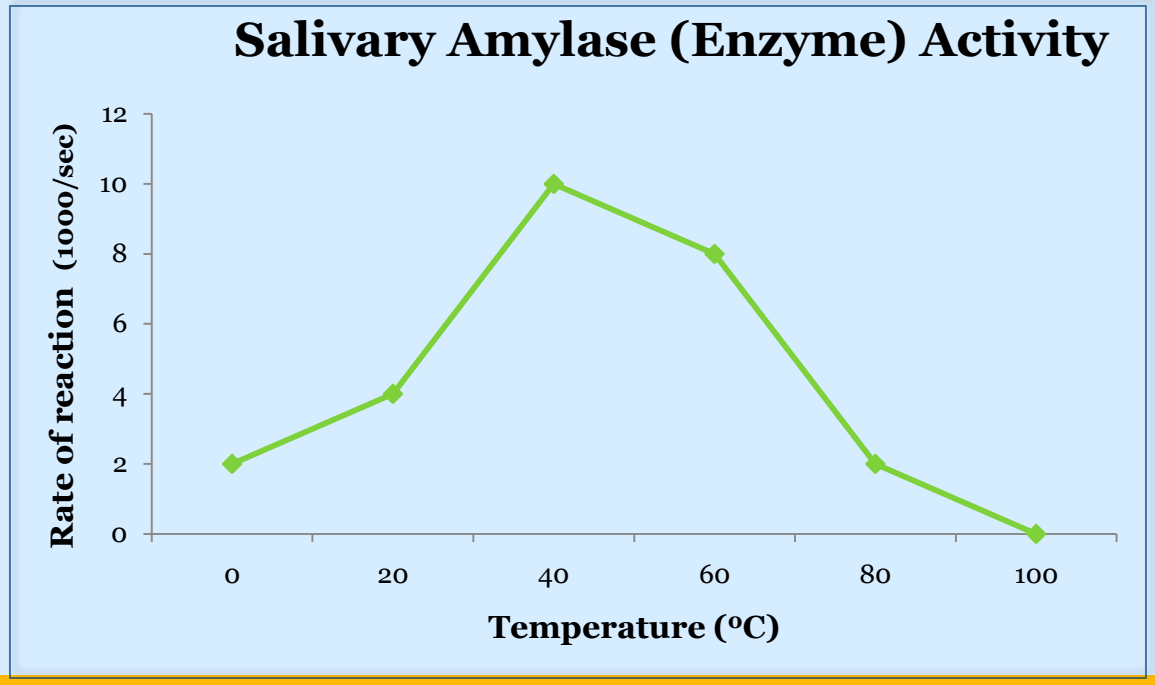
Starch $\xrightarrow{\text{salivary amylase}}$ **Maltose**

End of Reaction:

- Reaction is over when **last 3 drops** are **negative** for starch → amber with no purplish/black present.
- Count the # of drops it took for starch to be broken down to maltose.
- Total elapsed time = # of drops x 15 sec./drop
- Reaction rate = 1000/total elapsed time (seconds)
- Repeat this procedure for all temperatures.

Sample Results:

- Graph rate of reaction (y-axis) vs. temperature (x-axis).
- Write an explanation of salivary amylase activity based on your results. Be sure to include optimal temperatures (why?) and temperature range for this enzyme (and why?). What happens at extremely high temps? Did you get expected results? Explain. What about AMY1 genetics?



Post-Lab Assignment:

- Hand in the following at the beginning of next lab:
- Graph of Enzyme Results (Rate vs. Temperature) with a short (typed) written explanation.
- Also include raw data & calculations (e.g. 20°C results: 9 drops x 15 sec intervals = 135 seconds).

RAW DATA	Total Drops	15 or 30 sec. intervals?	Total Time (sec.):	Reaction Rate (sec.):
0°C	20	15	300	3.333
20°C	12	15	180	5.555
40°C	8	15	120	8.333
60°C	10	15	150	6.666
80°C	30	15	450	2.222
100°C	35	15	525	1.90

Any Questions?

- Before you begin: Instructors will demonstrate the timing/droplet placement. **This is super important for experiment to work!**
- Partner #1: Start labeling your starch test tubes & put them into respective water baths.
- Partner #2: start collecting saliva.