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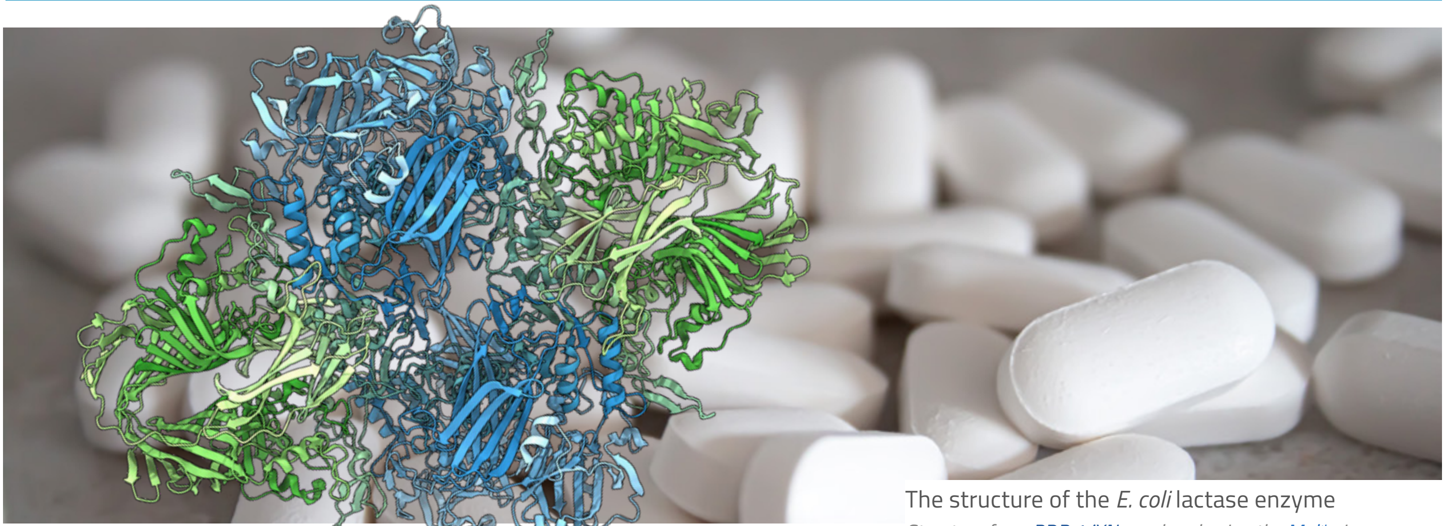

The structure of the *E. coli* lactase enzyme
Structure from PDB:1JYN, rendered using the Mol viewer*

Explore enzymes and the science of lactose intolerance using lactase tablets

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Lactase tablets for managing lactose intolerance can be used in the classroom to explore the biochemistry of sugars and the properties of enzymes.

Background:
Lactose intolerance is a common phenomenon,[1] based on the absence or insufficient production of the digestive enzyme β-galactosidase (lactase).[2] Affected people are unable to break down the disaccharide lactose (figure 1, top) into its monosaccharide subunits, glucose (Glc) and galactose, and they may suffer from diarrhoea and flatulence[3] after eating lactose-containing foods. In response, a growing market for lactose-free foods has emerged. Alternatively, affected individuals may choose to take lactase in tablet form to help them digest lactose-containing foods. In milk alternatives, such as oat milk, the sugar maltose (figure 1, bottom) can be found. The minor structural differences between lactose and maltose are the spatial position of a single hydroxy group and the type of glycosidic bond between the monosaccharide units (figure 1, red highlights).

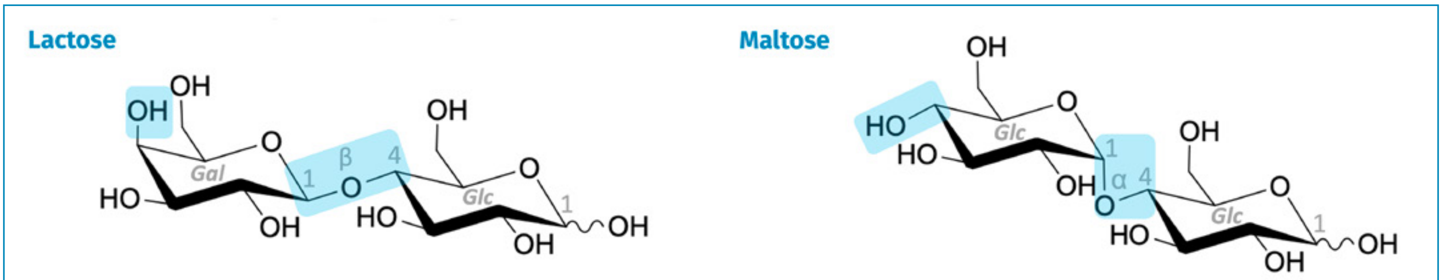

Figure 1: Molecular structures of the disaccharides lactose (left) and maltose (right), shown here as chair conformations. Respective monosaccharide units (galactose, Gal; glucose, Glc), carbon-atom numbers, and types of glycosidic bond are indicated in grey; structural differences are highlighted in red; and wavy bonds indicate reductive or ring-opening capabilities.
Image courtesy of the author

Here, we present activities addressing the curricular topics enzymology and sugars using scientific methods that are suitable for students aged 14–18 years. Students investigate the activity of lactose tablets on two structurally closely related sugars, lactose and maltose, in solution as well as in whole milk and oat milk. These experiments demonstrate that the enzyme lactase is specific to the substrate lactose, while maltose cannot be broken down. Additional experiments address why lactase-intolerant individuals should not take lactase tablets on an empty stomach, thus revealing the pH dependence of enzymes. In addition, students can investigate the effect of temperature on enzymes and why these drugs must be protected from heat, thereby exploring the impact of enzymes, or lack thereof, on everyday life.

The Fearon test[4] is used to detect sugars throughout the experiments since it enables easy, safe, and colourful differentiation of sugars.[5]

Preparation for all activities

1. Prepare Fearon's reagent by dissolving 0.5 g methylammonium chloride and 2 g sodium hydroxide in 100 ml water. The solution can be stored in an amber glass bottle and has a shelf life of approximately one year. Upon formation of suspended particulate matter, the solution should be discarded. The bottle label should include GHS pictogram GHS05 (metal corrosive) and the hazard warning caution.[6]
2. Sugar solutions and liquids: whole milk and oat milk can be sourced from grocery stores. The amounts of sugar solutions prepared beforehand should be adapted to the expected number of students; aliquots should be provided for each group.
3. Prepare the water baths by preheating sufficiently sized vessels to 65°C.

Activity 1: Detecting different sugars using Fearon's reagent

In this preparatory activity, students are introduced to sugar detection using Fearon's reagent on various sugar solutions as well as whole milk and oat milk.

The reduction of 1,4-linked disaccharides, such as maltose, lactose, and cellobiose, can be detected with Fearon's reagent through the formation of a bright-red dye. In contrast, monosaccharides, such as glucose, will produce a yellow dye, while β-1,2-linked disaccharides, such as sucrose, will not lead to a colour change. The mechanism of dye formation with Fearon's reagent is not fully explained, to date; however, there are well-founded hypotheses supported by a growing body of empirical evidence.[7]

This activity takes about 15 minutes.

Safety notes

- Whole milk and oat milk used in experiments are no longer suitable for consumption.
- Danger of scalding when using a water bath (65°C).
- Eye protection should be worn when using strongly alkaline Fearon's reagent.
- Contact of chemicals with the skin and eyes should be avoided in general. In case of contact, affected areas should be rinsed thoroughly with water.

Materials

Equipment per group:

- 6 test tubes
- 1 test tube stand
- 1 large beaker (e.g., 600 ml) to serve as a water bath
- 1 thermometer (up to 100°C)
- 1 heating plate, if necessary
- 7 dropping pipettes
- Waterproof pencil
- Aliquots of solutions
- Worksheet 1

Chemicals:

- Lactose solution (1% w/w)
- Maltose solution (1% w/w)
- Glucose solution (1% w/w)
- Sucrose solution (1% w/w)
- Oat milk
- Whole milk
- Fearon's reagent

Procedure

1) Label the test tubes with numbers 1–6, or three-letter abbreviations for sugars (Lac, Mal, Glc, Suc) and "whole" or "oat" for milk products (figure 2, top), using a waterproof pencil. Then add the following solutions using dropping pipettes: 1) lactose solution, 2) maltose solution, 3) glucose solution, 4) sucrose solution, 5) whole milk, and 6) oat milk.

2) Add 1 mL of Fearon's reagent each test tube with a dropping pipette and thoroughly swirl to mix.
3) Place 6x test tubes in a water bath preheated to 65°C (figure 2, right).
4) Incubate the solutions for approximately 5 minutes.
5) Observe and document the results.

Results

Observations:

Test tubes 1 (Lac), 2 (Mal), 5 (whole), and 6 (oat) are red in colour.
Test tube 3 (Glc) is yellow in colour.
Test tube 4 does not appear to change colour.

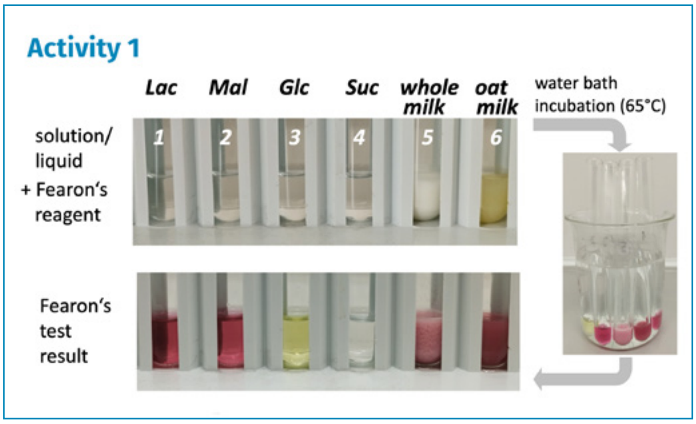

Figure 2: How to differentiate disaccharides, such as lactose and maltose, from monosaccharides, such as glucose and sucrose
Image courtesy of the author

Interpretation: Experimental results show that 1,4-linked disaccharides (lactose, maltose) can be detected using Fearon's reagent by a red colour. One or both of these sugars are present in both whole and oat milk. In addition, glucose (and other reducing monosaccharides) can be detected in solution by the formation of a yellow dye.

Activity 2: Investigating lactase activity on different sugars, whole milk, and oat milk

The Fearon test is used as a tool to investigate the activity and substrate specificity of the enzyme lactase, which is taken in tablet form as a food supplement by lactose-intolerant individuals. Specifically, in this activity, we explore the effects of lactase on two structurally related sugars, lactose and maltose (figure 1), as well as on whole milk and oat milk.

This activity takes about 20 minutes.

Safety notes

- Lactase tablets, whole milk, and oat milk used in experiments are no longer suitable for consumption.
- Danger of scalding when using a water bath (65°C).
- Eye protection should be worn when using strongly alkaline Fearon's reagent.
- Contact of chemicals with the skin and eyes should be avoided in general. In case of contact, affected areas should be rinsed thoroughly with water.

Materials

Equipment per group:

- 4 test tubes
- 1 test tube stand
- 2 large beakers (e.g., 600 ml) to serve as water baths
- 2 thermometers (up to 100°C)
- 1 heating plate, if necessary
- 5 dropping pipettes
- Waterproof pencil
- 1 stopwatch
- 1 mortar and pestle
- 1 small beaker
- Aliquots of liquid chemicals
- Worksheet 2

Chemicals:

- Lactose solution (4% w/w)
- Maltose solution (4% w/w)
- Oat milk
- Whole milk
- Fearon's reagent (see extension activity)
- Lactase express tablets (12 000 FCC ALU (Food Chemical Codex[1]), effective from the first minute)

Notes

It is important to use tap water and not distilled water to prepare enzyme solutions, as it contains certain ion species, for example, calcium, which can act as an enzyme cofactor.

Procedure

1) Read the introduction to lactase in the student worksheet.
2) Label the test tubes with numbers 1–4, or three-letter abbreviations for sugars (Lac, Mal) and whole or oat for

milk products (figure 3, top), using a waterproof pencil. Then add 1 ml of the following solutions using dropping pipettes: 1) lactose solution, 2) maltose solution, 3) whole milk, and 4) oat milk.
3) Using a mortar and pestle, pulverize a lactase express tablet and transferred it to a small beaker.
4) Add 10 mL of tap water using a dropping pipette, followed by mixing to dissolve the pulverized lactase tablet.
5) Add 1 mL of lactase solution from the small beaker to each of the test tubes using a dropping pipette and shake gently to mix.
6) Place the test tubes in a preheated water bath (38°C) for 5 minutes, simulating human body temperature.
7) Add 1 mL of Fearon's reagent to each test tube with a dropping pipette and thoroughly swirl to mix.
8) Place test tubes in a water bath preheated to 65°C.
9) Incubate the solutions for approximately 5 minutes.
10) Observe and document results, for example, on the [extension activity worksheet](#).

Observations: In test tubes 1 (Lac) and 3 (whole milk), a yellow colour appears during incubation in the water bath following the addition of Fearon's reagent, while a red dye forms in test tubes 2 (Mal) and 4 (oat milk; figure 3, compare top and bottom images).

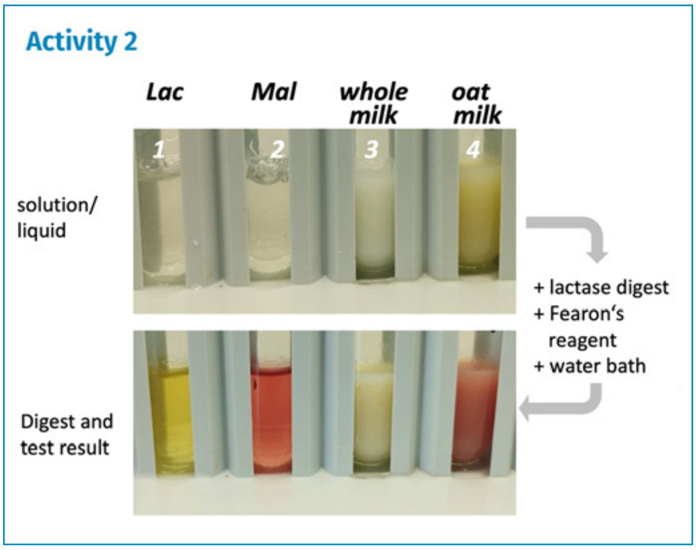

Figure 3: Using the Fearon test to detect lactase tablet activity in sugar solutions and liquid foods
Image courtesy of the author

Interpretation: The absence of red dye in test tubes 1 (lactose) and 3 (whole milk) indicates the disappearance of 1,4-linked disaccharides from these liquids, while the appearance of a yellow dye points to the presence of glucose, a known product of lactose breakdown (figure 1, top). These results demonstrate the successful breakdown of milk sugar (lactose) to glucose and galactose by lactase.

In contrast, a red dye still forms in test tubes 2 (maltose) and 4 (oat milk) following lactase treatment. This indicates that maltose cannot be digested by lactase, thus demonstrating the substrate specificity of this enzyme and that oat milk contains maltose, or a different 1,4-linked disaccharide, that cannot be digested by lactase.

Activity 3: Why lactase tablets should not be taken on an empty stomach

Instructions on the packaging of lactase tablets typically warn against taking the drugs on an empty stomach. In this activity, we scrutinize this disclaimer experimentally. Lactase ingested in tablet form has to pass through the human stomach, an acidic environment that contains hydrochloric acid at differing concentrations, depending on the filling level. Therefore, we simulate the exposure of lactase to 'gastric acid' in an empty stomach through preincubation in hydrochloric acid and assess its digestive performance following this treatment.

This activity takes about 25 minutes.

Safety notes

- Whole milk and oat milk used in experiments are no longer suitable for consumption.
- Danger of scalding when using a water bath (65°C).
- Eye protection should be worn when using strongly alkaline Fearon's reagent.
- Contact of chemicals with the skin and eyes should be avoided in general. In case of contact, affected areas should be rinsed thoroughly with water.

Materials

Equipment per group:

- 4 test tubes
- 1 test tube stand
- 2 large beakers (e.g., 600 ml) to serve as water baths
- 2 thermometers (up to 100° C)
- 1 heating plate, if necessary
- 6 dropping pipettes
- Waterproof pencil
- 1 stopwatch
- 1 mortar and pestle
- 2 small beaker

- Aliquots of liquid chemicals
- Worksheet 3

Chemicals:
- Lactose solution (1% w/w)
- Maltose solution (1% w/w)
- Oat milk
- Whole milk
- Fearon’s reagent
- Lactase express tablets (12 000 FCC ALU (Food Chemical Codex[1]), effective from the first minute)
- Hydrochloric acid (c=0.1 mol l^{-1})

Procedure

1) Label the test tubes with numbers 1–4, or three-letter abbreviations for sugars (Lac, Mal) and whole or oat for milk products (figure 4, top), using a waterproof pencil. Add 1 ml of the following solutions using dropping pipettes: 1) lactose-solution, 2) maltose solution, 3), whole milk, and 4) oat milk.
2) Using a mortar and pestle, pulverize a lactase express tablet and transfer to a small beaker.
3) Add 10 ml of 0.1 M solution of hydrochloric acid to each tube and incubate the solutions for 3 minutes. This hydrochloric acid concentration corresponds to the acidity of an empty stomach.
4) Add 1 mL of acid-treated lactase solution from the small beaker to each of the test tubes using a dropping pipette and swirl gently to mix.
5) Place the test tubes in a preheated water bath (38 °C) for 5 minutes, simulating human body temperature.
6) Add 1 mL of Fearon’s reagent to each test tube with a dropping pipette and thoroughly swirl to mix.
7) Place test tubes in a water bath preheated to 65 °C.
8) Incubate the solutions for approximately 5 minutes.
9) Observe and document results.

Notes

The molecular structure of the red dye that is formed using Fearon’s reagent varies according to the pH value. Hence, the shade of red observed in this experiment may differ from that obtained in previous experiments carried out under more alkaline environments (extension activity and Activity 1).

The assays in tubes 2 (Mal) and 4 (oat milk) serve as negative controls, as previous experiments (Activity 1) demonstrated the inability of lactase to digest the disaccharides present in these samples.

Observation: Unlike in a previous experiment (Activity 2) without preincubation with hydrochloric acid, the formation of a red dye is observed in all four assays (figure 4). These results are identical to those obtained for the Fearon tests on lactose, maltose, whole milk, and oat milk without prior lactase digestion (figure 2, bottom).

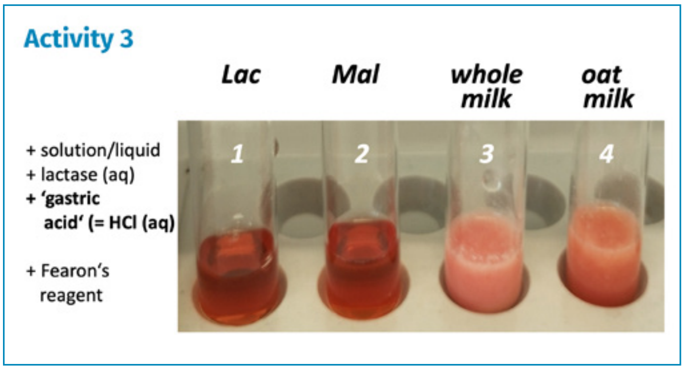

Figure 4: Acid, like that found in the stomach, inactivates lactase
Image courtesy of the author

Interpretation: Enzyme activity is highly dependent on the environmental pH value. By dissolving the lactase tablet in 0.1 M hydrochloric acid, we simulate the ingestion of lactase tablets on an empty stomach. The persistence of red-dye formation using Fearon’s reagent and the absence of yellow-dye formation – indicating glucose formation as a result of lactose breakdown (as observed during a previous experiment without acid pretreatment; figure 3) – demonstrate the loss of lactase activity under low-pH conditions like those in an empty human stomach. This can be explained by changes to the 3D structure of the enzyme through acid denaturation.

Optional extension activity

Instructions on the packaging of lactase tablets typically also warn against exposing the drugs to heat, for example, taking them with hot drinks. As an optional extension activity, you can explore the influence of heat on enzyme activity. Details are given in the supporting material. «

References

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