JOURNAL OF CHEMICAL EDUCATION

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Investigating the Hydrolysis of Starch Using α-Amylase Contained in Dishwashing Detergent and Human Saliva

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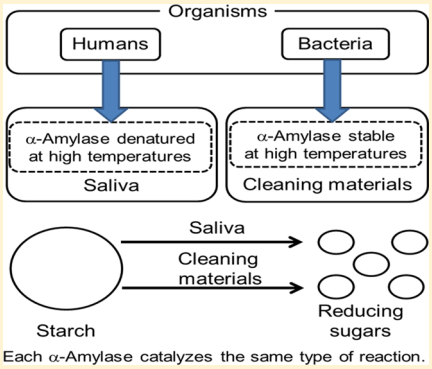
Supporting Information

ABSTRACT: Although saliva has commonly been used to teach about digestion by organisms, the phenomenon of digestion is actually caused by enzymes as catalytic substances. This activity explores the hydrolysis of starch by α-amylase in cleaning materials as well as a comparison with the similar reaction using human saliva. The fact that the α-amylase contained in the cleaning materials catalyzes hydrolysis of starch without saliva may remind students of the aspect of an enzyme as a chemical material. In addition, discovering the difference in the reactions between cleaning materials and human saliva at higher temperatures may be a useful educational approach for explaining the stability and denaturation of enzymes in an engaging way that deepens students' understanding.



Each α-Amylase catalyzes the same type of reaction.

KEYWORDS: *Elementary/Middle School Science, High School/Introductory Chemistry, Biochemistry, Organic Chemistry, Hands-On Learning/Manipulatives, Aldehydes/Ketones, Carbohydrates, Catalysis, Enzymes, Food Science*

INTRODUCTION

Incorporating Enzyme Reactions in the Science Curriculum

The latest Japanese Guidelines[1] of Education propose four core ideas, energy, particles (or matter), life, and earth, which correspond to the subjects physics, chemistry, biology, and geology, respectively. These core ideas[2] make the borders between the conventional science subjects flexible.[3,4] These core ideas also foster natural phenomena to be seen from multidisciplinary points of view.[3,4]

For instance, compare the fact that human saliva digests[5−7] starch with the fact that an enzyme (α-amylase) in saliva catalyzes a chemical reaction, the hydrolysis of starch. Because the learning item, the enzyme, is located in the border between the core ideas of life and matter, which involves the concept of molecules, it may be used for understanding both of these core ideas. An enzyme is not alive, but it is a component of life. A living thing needs catalysis by enzymes, which are mainly composed of proteins as matter. These viewpoints suggest that enzymes seem to be in the border between life and matter.

Saliva has been used[5−7] as a typical educational material for instruction on the relationship between digestion and hydrolysis of starch. The use of saliva is an example of digestion as an action of life; however, excessive stress on digestion may lead to a weakening of the importance of chemical reactions. A stronger foundation in chemical reactions may be achieved by changing the focus to everyday products that contain digestive enzymes. Some everyday products contain hydrolytic enzymes. On the other hand, many kinds of enzyme-containing materials are present in everyday life. Some materials contain hydrolytic enzymes extracted from bacteria, such as toothpaste and cleaning materials for washing clothes or dishes. Commercially available cleaning materials that list enzymes as ingredients may contain enzymes like α-amylase and proteases. These cleaning materials may be used to catalyze chemical reactions in the classroom or laboratory settings in schools. However, very few educational uses of enzyme-containing materials from everyday life have been reported.[8,9] We highlight the commercial, enzymatic digestion of starch as one such example from everyday life.

Starch[10] is composed of a linear polymer, α-amylose, and a branched polymer, amylopectin. α-Amylose has several thousand glucose residues linked with α(1→4) bonds. Amylopectin also has thousands of glucose residues linked with α(1→4) bonds and branches at every 24−30 residues[10] with α(1→6) bonds. α-Amylase randomly hydrolyzes all the α(1→4) glucosidic bonds of starch except its outermost bonds and those next to branches.[10] α-Amylase yields many chain numbers of reducing oligosaccharides until reaching the glucose dimer, maltose (n = 1) (Figure 1). The resulting oligosaccharides tautomerize through an aldehyde form to give a mixture of α- and β-anomers. Although a starch molecule does not have enough reducing ends to test positive to Benedict's reagent,[7,11] α-amylase hydrolyzes starch into many oligosaccharide molecules, each with a reducing end. Therefore,

Received: August 18, 2015
Revised: March 18, 2016

DOI: 10.1021/acs.jchemed.5b00545
J. Chem. Educ. XXXX, XXX, XXX−XXX

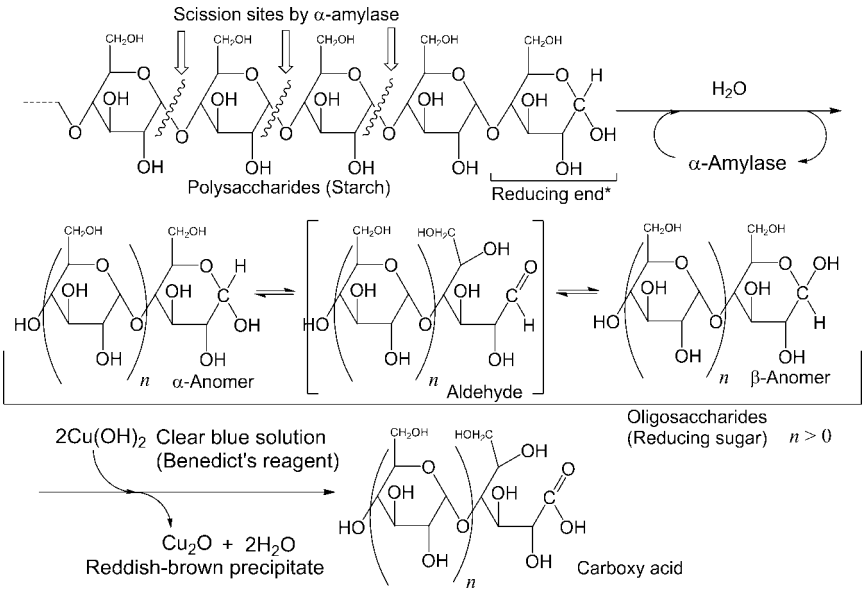


Figure 1. Scission points of starch by amylase and products: reducing oligosaccharides. *Full-length starch does not contain enough reducing equivalents to test positive to Benedict's reagent before hydrolysis.

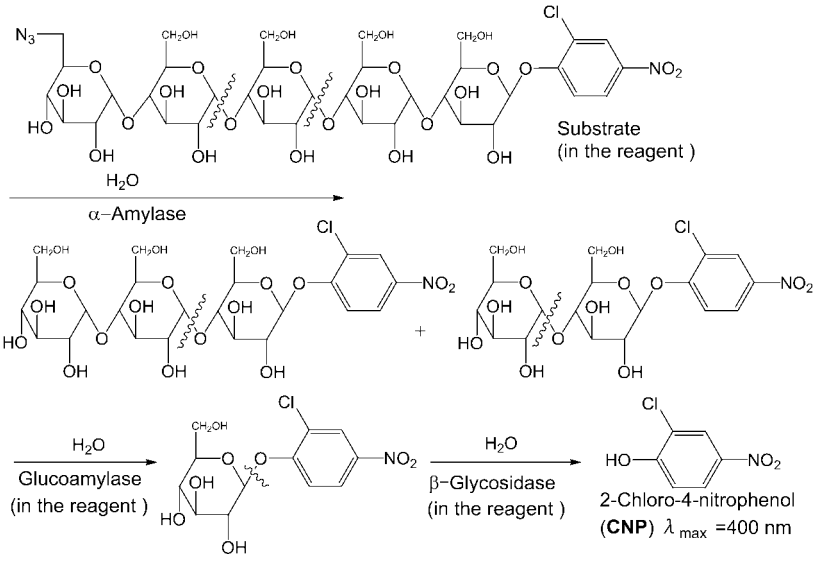


Figure 2. Determination of α-amylase activity in cleaning materials and saliva. A colorimetric kit involving a synthetic substrate was used for determining the enzyme activity.

the reaction solutions of starch with α-amylase are positive to Benedict's reagent.[7,11]

This study addresses the hydrolysis of natural and synthetic starch substrates by the α-amylase found in either cleaning materials or human saliva. Differences in enzyme reactivity are measured using Benedict's reagent[7,11] and a separate colorimetric kit.[12]

MATERIALS AND METHODS

Several kinds of starch, rice powder, cornstarch, potato starch, "kuzu" starch (devil's tongue), wheat flours (weak, medium, and strong), and pasta, were purchased for the reactions. Of these, only rice powder was not a suitable substrate for visualizing the reactions using cleaning materials because rice powder already has the ability to react with Benedict's reagent before the hydrolytic reactions with α-amylase. Wheat flours like gluten and other intolerances should be considered prior to use. Choice criterion of starch materials is mentioned in the Supporting Information.

Each starch sample (0.025 g) was heated with water (2 mL) for 5 min in a polypropylene test tube in an aluminum heat bath set at 110 °C to produce a starch gel solution, which was cooled to room temperature before the reactions with solutions of the cleaning materials. Three kinds of cleaning materials, Charmy Crysta Clear Gel (C) (Lion Corporation, Tokyo, Japan), Joy (J) (Procter & Gamble, Kobe, Japan), and Kyukyutto (K) (Kao Chemicals, Japan), were diluted with distilled water to form 0.3, 1.7, 3.3, 17, and 25% (w/v) solutions. Distilled water was used as a control. Each solution of cleaning material (2 mL) was mixed by vortexing with a starch sample solution in the same polypropylene test tube, which was capped with a screw cap and then heated for 10 min in an aluminum bath set at a constant temperature (30, 50, 70, and 100 °C). After a cooling period with flowing water to room

DOI: 10.1021/acs.jchemed.5b00545
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temperature as the starting condition for Benedict's reaction, each reaction solution was quenched with 1 mL of Benedict's reagent[7,11] and heated for 10 min in an aluminum bath set at 110 °C to determine the level of reducing sugar. The temperature, set at 30, 50, 70, 100, and 110 °C, showed the actual reaction temperature as 30, 45, 62, 86, and 95 °C. The reaction is shown in Figure 1. Benedict's reagent was made as follows: anhydrous sodium carbonate (10.0 g) and sodium tartrate (17.3 g) were dissolved in 80 mL of hot distilled water to give a solution. $CuSO_4 \cdot 5H_2O$ (1.73 g) was dissolved in 10 mL of distilled water. These solutions were mixed and then diluted with distilled water to give 100 mL of Benedict's reagent. The detailed procedure can be seen in the Supporting Information.

A cotton-tipped applicator was placed in one student's mouth to collect saliva, then removed and immersed in distilled water (2 mL) and shaken for 1 min. The extracted saliva solution was treated as above and used for comparison with the reactions using solutions of cleaning materials and Benedict's reagent.

As shown in Figures 2 and 3, a synthetic substrate, 2-chloro-4-nitrophenol 6^5-azide-6^5-deoxy-β-maltopentaoside, was hydro-

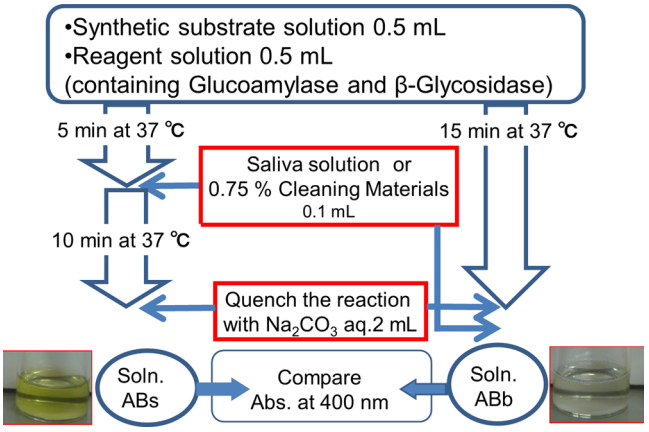

Figure 3. Procedure for determining α-amylase activity on synthetic substrate using the Kikkoman kit. ABb, absorption of blank at 400 nm; ABs, absorption of sample at 400 nm.

lyzed by the action of α-amylase to afford 2-chloro-4-nitrophenol derivatives of glucose dimer and trimer, which were hydrolyzed by glucoamylase and then β-glycosidase to release 2-chloro-4-nitrophenol (CNP).[12] After the reaction was quenched with sodium carbonate solution, absorbance of CNP at 400 nm was measured with a Shimadzu spectrophotometer UV-1200 (Shimadzu, Kyoto, Japan). For determining α-amylase activity by the method described above, a colorimetric kit (Kikkoman Corporation) was used. The enzyme activity of α-amylase is given as follows:

$$\text{Enzyme activity} = (\text{ABs} - \text{ABb}) \times 0.179 \times \text{Df}$$

where ABs is absorption of sample solution, ABb is absorption of blank solution, and Df is dilution factor. Activity of C, J, and K was measured for 0.75% aqueous solutions (v/v) of cleaning materials; the activity of saliva was measured for the solution of 2 mL of aqueous solution immersed by a licked cotton-tipped applicator containing 0.123 g of saliva diluted 10-fold. Df's were 133 for cleaning materials and 17.1 for saliva.

RESULTS AND DISCUSSION

Concentration of Cleaning Materials

Suitable concentrations of cleaning materials for hydrolysis of starch were examined at 30 °C using weak, medium, and strong wheat flours, and pasta. Figure 4 shows the results of Benedict's

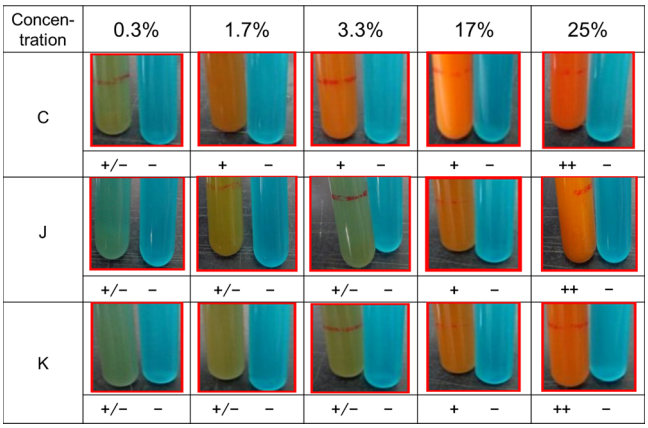

Figure 4. Benedict's reactions after α-amylase hydrolysis of weak flour using the following cleaning materials: Charmy Crysta Clear Gel (C), Joy (J), and Kyukyutto (K). Concentration: Initial concentration of cleaning materials solution before mixing with starch solution. In each square, left and right sides are with and without cleaning materials, respectively. Under each square, (−), no reaction; (+/−), weak reaction; (+), strong reaction; (++), very strong reaction. Experiments were conducted by the authors.

reactions after enzymatic reaction of weak flour using the cleaning materials Charmy Crysta Clear Gel, Joy, and Kyukyutto. In the case of Charmy Crysta Clear Gel (C), 0.3% of the cleaning material showed a green rather than a reddish-brown color. Reddish-brown color is consistent with the presence of increased reducing sugars, resulting from increased starch hydrolysis, while the green color is closer to the blue control and is consistent with fewer reducing sugars, resulting from less starch hydrolysis.

However, Kyukyutto (K) and Joy (J) showed different results, which are consistent with K and J being less hydrolytically active than C. Concentrations of 0.3%, 1.7%, and 3.3% did not show a reddish-brown color, whereas higher concentrations showed a color change to reddish-brown. Medium flour, strong flour, and pasta showed similar results (data not shown) to those of weak flours. Cornstarch, potato starch, and "Kuzu" starch showed a color change to brown or reddish-brown using 17% and 25% at 20 °C for the cleaning materials except of Kyukyutto, which showed green or pale brown in most cases (Figure 5).

Temperature of the Reactions

The hydrolysis of potato starch at different temperatures is shown for the results of Benedict's reaction in Figure 6. After the hydrolysis set at 30, 50, 70, and 100 °C, the results using the three kinds of cleaning materials showed a reddish-brown color with Benedict's reagent. However, the hydrolysis reactions using saliva at higher temperatures did not show a reddish-brown color. The result may be explained by the difference in the thermal stability between the two α-amylases. The α-amylase in saliva was denatured at higher temperatures to lose activity, while the α-amylase in cleaning materials resisted heating.

DOI: 10.1021/acs.jchemed.5b00545
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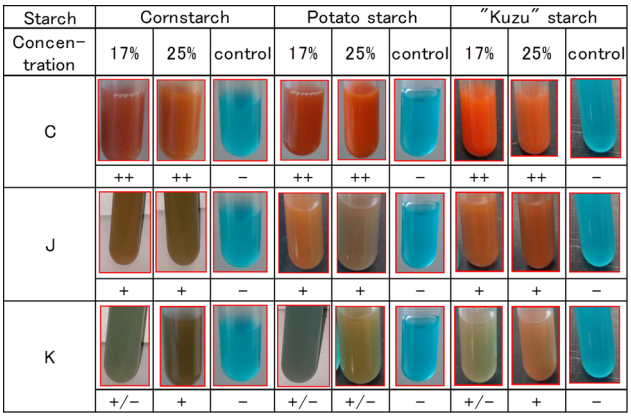

Figure 5. Benedict's reactions after α-amylase hydrolysis of cornstarch, potato starch, and "Kuzu" starch using the following cleaning materials: Charmy Crysta Clear Gel (C), Joy (J), and Kyukyutto (K). Concentration: Initial concentration of cleaning materials solution before mixing with starch solution. Control is the result without cleaning materials. Under each square, (−), no reaction; (+/−), weak reaction; (+), strong reaction; (++), very strong reaction. Experiments were conducted by the authors.

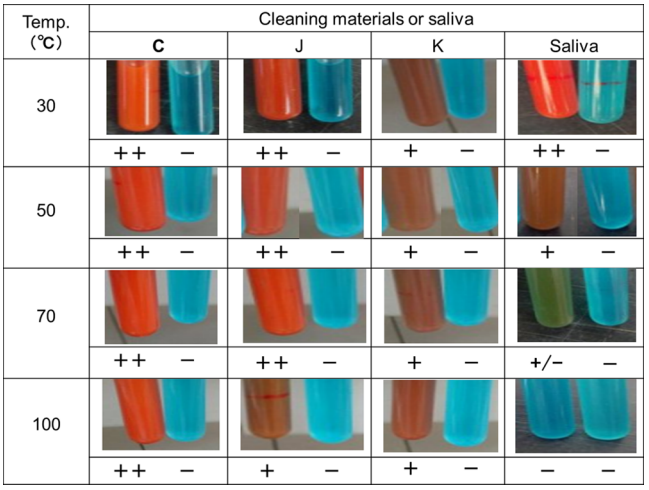

Figure 6. Dependence on reaction temperature of results using different enzyme sources. Temp., reaction temperature; C, Charmy Crysta Clear Gel; J, Joy; K, Kyukyutto. Concentration of cleaning materials: 17%. (−), no reaction; (+/−), weak reaction; (+), strong reaction; (++), very strong reaction. Experiments were conducted using potato starch by the authors.

Measurement of Amylase Activity with Synthetic Substrate

Figure 7 compares the α-amylase activity of saliva and the cleaning materials used. Charmy Crysta Clear Gel shows a higher activity than Kyukyutto and Joy. The value of enzymatic activity was consistent with the results with Benedict's reagent. However, there seem to be some conflicts in the results between Benedict's reaction and colorimetry using the Kikkoman kit. Joy was more active than Kyukyutto in Benedict's reaction, while Kyukyutto was more active than Joy using the Kikkoman kit. The conflicts might be caused by the reddish-brown color in Benedict's reaction mixed with the whitish turbidity of Kyukyutto, which does not give a vivid reddish-brown color. Since only Kyukyutto contains *N,N′*-bis(carboxymethyl)-L-glutamate, it may chelate to Cu(II) ion to disturb the formation of Cu_2O.

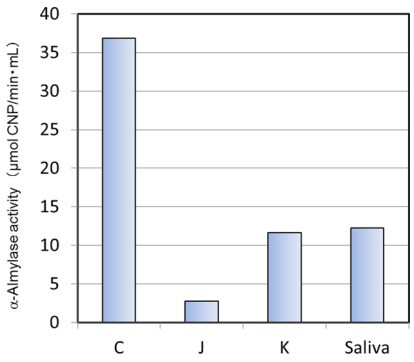

Figure 7. Enzyme activity of cleaning materials: Charmy Crysta Clear Gel (C), Joy (J), Kyukyutto (K), and saliva solution. Activity of C, J, and K is calculated for 0.75% aqueous solutions (v/v) of cleaning materials; activity of saliva is calculated for 2 mL aqueous solution containing a licked cotton-tipped applicator. Experiments were conducted by the authors.

Implication for Classroom Science in Secondary Schools

This research demonstrates that enzyme activity can be seen in everyday industrial products as well as human secretion products such as saliva, which has been used as a typical educational material for teaching digestion. The commonality of an enzyme existing in cleaning materials and in saliva can introduce the concept that an enzyme is a "matter" catalyzing chemical reactions as well as being from "life". It also shows that matter extracted from life can be used for industrial products in everyday life. Although enzymes in cleaning materials and saliva are different in their stability to heat, the difference may be useful for explaining the same activity but the different sources of enzymes. The results of the research may be applicable to many cases of classroom and laboratory situations.

In the classroom, for example, the difference in thermostability between α-amylase from saliva and that in cleaning materials will lead to the thread of making sweeteners from corn syrup in industry. The sweetener-making process needs three enzymes in the three steps, in which the first step is the hydrolysis of starch in corn syrup[13] at higher temperatures using thermostable α-amylase from bacteria. Meanwhile, α-amylase from saliva cannot be used for this purpose because it is unstable at higher temperatures.

The Supporting Information contains a description of a typical experimental procedure, which may be modified according to the circumstances. In particular, the heating of reactions can be simply changed to a type using a water bath because it may be difficult for secondary schools to obtain aluminum heat baths. In such cases, comparing saliva with the cleaning materials also can be simplified to the two conditions at room temperature and in boiling water. The experiment with 20 students of a junior high school class was done using the simplified conditions in 2013. The experiments involved individual work by students, who used one of the cleaning materials solutions. It took about 40−50 min to finish all of the procedures from weighing cleaning materials through enzyme reactions to detection of reducing sugars.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00545.

DOI: 10.1021/acs.jchemed.5b00545
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A detailed experimental procedure for 2nd grade junior high school students (corresponding to 8th grade), simplified heating method, choice of starch sample (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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