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Cicada Shells as Chemical Education Resources to Study Functional Groups and Environmental Cycles

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Abstract Cicada shells are widely available and have been used to teach insect anatomy; their tactile nature is ideal for students who are reluctant to handle living insects. The main component of cicada shells is chitin, which has a polymer structure of *N*-acetylglucosamine that produces an amorphous gel in neutral aqueous solutions. Glucosamine has a reducing group (hemiacetal structure) and an amino group, and these functional groups can be detected with Benedict reagent and ninhydrin, respectively. This research proposes a procedure to detect the glucosamine structural features present in cicada shells and highlights the link between chemicals in the cicada shells and the environment. Cicada shells can thus be useful for teaching classes about chemicals and their role in the environment.

Keywords: *cicada shells, chitin, glucosamine, educational materials, environmental cycles*

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1. Introduction

"Cicada nymphs spend many years in the soil of the plant rhizosphere, where they feed solely on xylem fluid from plant roots" [1,2]. Cicada shells retain the shape of the last form of the nymph and are useful morphological materials for teaching science in elementary, secondary, and high school, as well as in teacher training courses [3]. The individual parts of cicada shells offer well-defined materials with which to clearly compare and study the common elements of insects. Cicada shells also offer tactile materials for students who are reluctant to handle living insects [3]. We have trialed the use of parts of cicada shells as readily available materials for science classes in Naruto University of Education. Students were able to cleave cicada shells with cutters to identify each component and confirm the textbook images of each part of the cicada shells [3]. Although this is an example of teaching material for the biological field of science, cicada shells may also be useful for teaching in the chemical field.

Cicada shells are mainly composed of chitin, which is the principal component of the exoskeletons of insects and crustaceans (crab, shrimp, lobster, sow bug, pill bug, etc.). Chitin [4,5] and chitosan [5,6] have been used as teaching materials in the chemistry field; for example, cicada exoskeletons and beetles were used for electroless deposition [4], chitosan was used to study adsorption of organic dyes [6], and both chitin and chitosan from lobster shells were used for bioplastics [5]. Cicada shells retain the shape of the cicada nymph for a long time under ambient conditions because the chemical building blocks are primarily composed of chitin [3] (Figure 1). However, upon contact with soil and/or harsh environmental factors, such as sunlight or physical actions, the shells change their structure, and the material degrades to other compounds. *Bacillus* [7,8] and *Streptomyces* [9,10] in the soil, as well as fungi [11], have chitinases, which hydrolyze insoluble chitin molecules in water to soluble oligomers.

We envisioned that we could use the hydrolysis of chitin to glucosamine or chitosan oligomer (Figure 1) to study their chemical structure, and that we could use the process of hydrolysis and detection as an educational aid for those teaching chemical science.

In this study, we selected hydrochloric acid-mediated hydrolysis to study the hydrolysis process of chitin (Figure 1) because, like cicada shells, hydrochloric acid is widely available in schools. We established hydrolysis conditions to decompose cicada shells and identified Benedict (Figure 2) and ninhydrin (Figure 3) as suitable reagents for the detection of reducing sugars and amino groups, respectively. This research demonstrates both the use of cicada shells as teaching materials in the chemistry field and leads to further discussions on environmental themes.

During acid-mediated hydrolysis, the number of hemiacetal and free amino groups will increase. At constant reaction times, the reaction mixtures can be analyzed with Benedict reagent for hemiacetal moieties and ninhydrin for free amino groups. This research provides robust methods for studying the reactive structures formed by acid-hydrolysis of cicada shells.

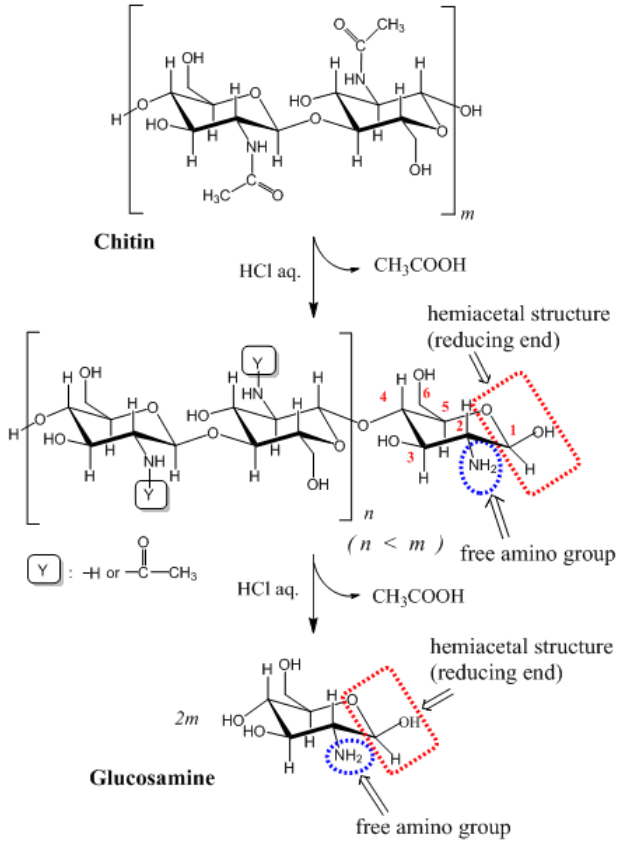

Figure 1. Acid-mediated hydrolysis of chitin through the formation of hemiacetal and free amino moieties to give glucosamine.

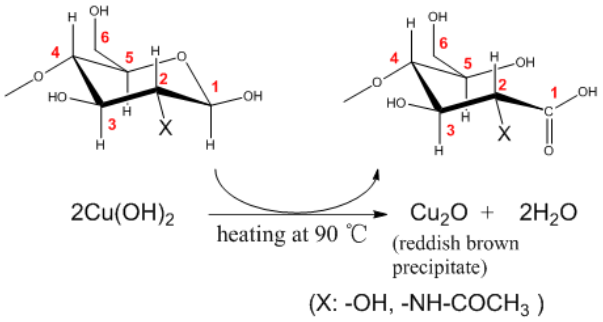

Figure 2. Benedict reaction with reducing sugars

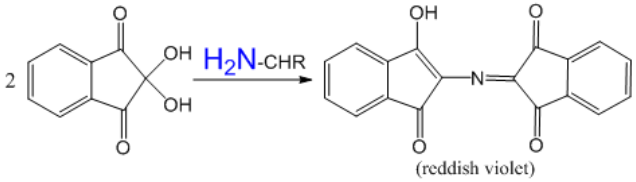

Figure 3. Ninhydrin reaction with compounds containing amino groups.

Chitin dissolves in 12 M HCl at room temperature, and the resulting solution can be diluted with water to give a suspension including gel particles. This suspension can be used as an example of a gel material. The obtained gel can be purified by dialysis with a cellulose membrane. The method is explained. When teachers are familiar with the procedures used for making a gel of chitin from cicada shells, they can find this approach a useful method (3.2.). The reaction data can be used to discuss the environmental cycles of chemicals passing through cicada shells. This research establishes a protocol in which cicada shells are used as chemical teaching materials and provides a method for modeling the relationship between cicada shells and the environment (see 3.3.).

2. Materials and Experiments

2.1. Cicada Shells

Shells of large brown cicadas (*Graptopsaltria nigrofuscata*) taken from the stems of trees in Naruto city, Tokushima, Japan, were used for this study. Cicada shells are composed of many parts of exoskeleton surface structure. Each part has a different physical consistency and a different solubility in acid. Thus, the thorax and abdomen were cut out from the structure of the cicada shell (Figure 4).

When a complete cicada shell was placed in 12 M HCl for about 24 h at room temperature, the front parts of the thorax and abdomen dissolved, whereas the other parts did not dissolve. The result suggested higher solubility and reactivity of these parts of the thorax and abdomen; thus only these parts (ca 10 mg from a complete structure of ca 150 mg) were used for an acid-hydrolysis reaction.

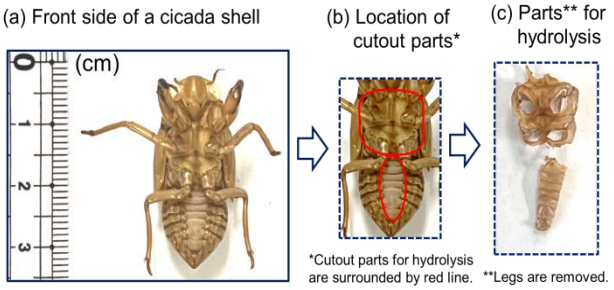

Figure 4. Dissection of cicada shells for the acid-hydrolysis reaction

2.2. Chemical Reagents

Standard chitin, glucosamine hydrochloride, glycine, ninhydrin, anhydrous sodium carbonate, sodium citrate dihydrate, copper sulfate pentahydrate, and concentrated hydrochloric acid (6 M HCl and 12 M HCl) were purchased from FUJIFILM Wako Pure Chemical Corporation, Japan.

Benedict reagent was prepared as follows: Anhydrous sodium carbonate (Na_3CO_3, 10.0 g) and sodium citrate dihydrate ($C_6H_5Na_3{\cdot}2H_2O$, 19.7 g) were mixed and dissolved in water (60 mL) to give a solution, to which was added a solution of copper (II) sulfate pentahydrate ($Cu_2SO_4{\cdot}5H_2O$, 1.73 g) in water (5 mL). The resulting mixed solution was diluted to 100 mL with water to afford a clear, blue solution, which was used as the Benedict reagent in this study.

Ninhydrin was prepared as a solution of ninhydrin (0.10 g), water (10 mL), and ethanol (40 mL). The resulting 0.2% ninhydrin solution was used as the ninhydrin indicator for spraying on glass plates for silica gel thin-layer chromatography.

2.3. Apparatus for Thermal Reactions

Reaction solutions for the hydrolysis of chitin and for monitoring the hydrolytic reactions were placed in glass containers and covered. Acid hydrolysis in the glass container was carried out by heating at 110 °C using an aluminum heating block (EYELA MG-2200). Benedict reaction at 90 °C was carried out with an aluminum heating block (ADVANTEC HTB 120DB, Japan).

2.4. Reaction Procedures

2.4.1. Acid Hydrolysis of Chitin and Cicada Shells

The front parts (10 mg) of the thorax and abdomen from a cicada shell and 2.0 mL of either 6 M or 12 M HCl were mixed in a glass tube with a cover. The reaction mixture was heated at either 110 °C or room temperature for a constant time.

2.4.2. Benedict and Ninhydrin Reactions with Chitin and Cicada Shells

For hydrolysis in 12 M HCl, the mixtures (0.40 mL) were removed from the reaction tube at constant time and neutralized with 5 M NaOH (0.96 mL) in a different glass tube. Benedict reagent (0.50 mL) was added to the solution, and the resulting solution was stirred and heated at 90 °C for 10 min. For hydrolysis in 6 M HCl, the mixtures (0.40 mL) were removed from the reaction tube at constant time and neutralized with 5 M NaOH (0.48 mL) in a different glass tube. Benedict reagent (0.50 mL) was added to the solution, and the resulting solution was stirred and heated at 90°C for 10 min.

A constant volume (2 μL) was taken out of the reaction solution with a glass capillary tube and spotted on a piece of silica gel plate. Aqueous solutions (1.0 mg/mL) of glucosamine hydrochloride and glycine were also spotted (2 μL) as standards. The plate was dried at room temperature, then ninhydrin was sprayed over the surface and the plate was heated on a plate heater.

2.4.3. Dialysis of Gel Formed from Cicada Shells

The dialysis procedure used for the gel made from cicada shells is described in the Results and Discussion (see 3.2.).

3. Results and Discussion

3.1. Benedict and Ninhydrin Reactions with Acid-Hydrolyzed Chitin and Cicada Shells

Standard chitin and cicada shells were separately hydrolyzed under three different conditions: (1) in 6 M HCl at 110°C, (2) in 12 M HCl at 110°C, and (3) in 12 M HCl at room temperature. The use of 6 M HCl at 110°C is known to result in complete acid hydrolysis of proteins; however, the use of 12 M HCl was also studied because concentrations higher than 6 M may be necessary for hydrolysis of chitin. The chosen temperatures were 110°C and room temperature (25°C). Although heating in 6 M and 12 M HCl are very harsh conditions, the room temperature reaction can be conducted in the classroom. After heating at different reaction times under conditions (1) to (3), the reaction mixtures were reacted separately with Benedict (3.1.1.) and ninhydrin (3.1.2.) reagents.

3.1.1. Detection of Reducing Sugars with Benedict Reagent

Standard chitin and cicada shells were separately heated in 6 M HCl at 110°C. The suspensions were extracted immediately after mixing at different reaction times. The extracted solutions were mixed with Benedict reagent and heated at 90 °C for 10 min. The results are shown in Figure 5.

Standard chitin and cicada shells were heated in 12 M HCl at 110°C. The suspensions were extracted immediately after mixing at different reaction times. Extracted solutions were mixed with Benedict reagent and heated at 90°C for 10 min. The results for chitin and cicada shells are shown in Figure 6.

Standard chitin and cicada shells were allowed to stand in 12 M HCl at 25 °C. The suspension was extracted to show the results using only standard chitin and cicada shells. The results are shown in Figure 7.

Comparing the results obtained with 6 M HCl at 110 °C, the hydrolyzed solutions of chitin gave a positive Benedict reaction after 8 h, whereas cicada shells gave a positive response even after 1 h (Figure 5).

A brown precipitate produced during the Benedict reaction of hydrolyzed cicada can be seen (Figure 5 (b)). The color might be caused by precipitation of cicada shells upon cooling the 6 M HCl from 110 °C. The cicada shells were originally brown in color (Figure 4).

As shown in Figure 6 (a), hydrolyzed solutions of chitin in 12 M HCl at 110 °C after 1 h revealed a clear red-brown precipitate. However, all the hydrolyzed solutions of cicada shells in Figure 6 (b) showed a brown precipitate. In the hydrolysis reaction of chitin, reducing ends were generated faster in 12 M HCl than in 6 M HCl at the same temperature (110°C).

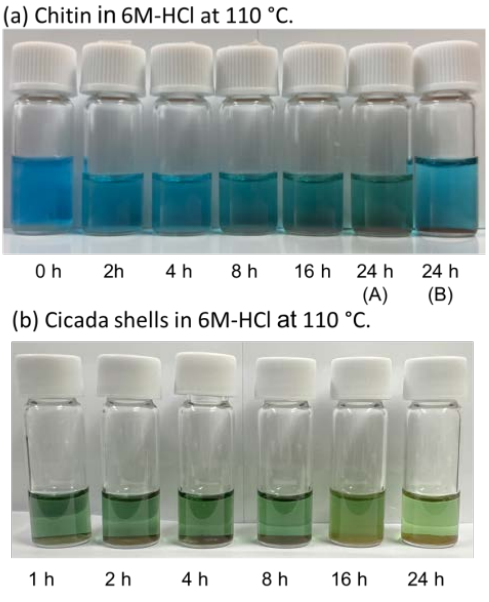

Figure 5. Benedict reaction of (a) standard chitin and (b) cicada shells hydrolyzed in 6 M HCl at 110 °C. Using Benedict: 0.50 mL for 24 h (A); 1.00 mL for 24 h (B).

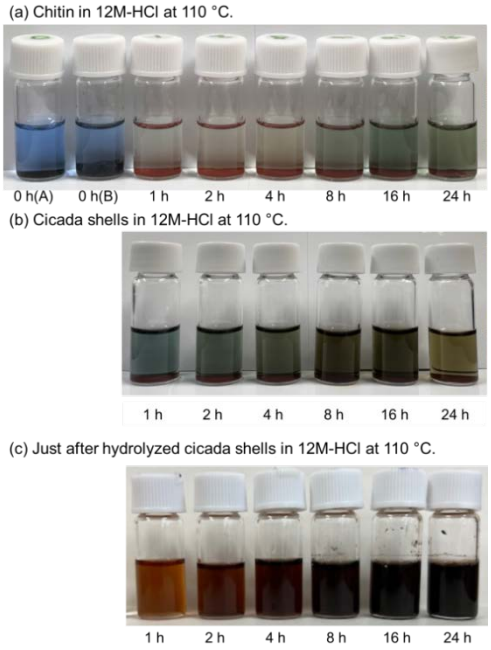

Figure 6. Benedict reaction of (a) standard chitin and (b) cicada shells hydrolyzed in 12 M HCl at 110 °C. (c) Acid-hydrolysis of cicada shells in 12 M HCl at 110 °C before the Benedict reaction. 0 h (A): without chitin; 0 h (B): with chitin.

Figure 6 (c) shows the hydrolyzed solutions of cicada shells in 12 M HCl at 110 °C before the Benedict reaction. All the samples contained a brown precipitate before they were neutralized and reacted with the Benedict reagent. This observation confirmed that the brown precipitates observed after the Benedict reactions were formed in the hydrolyzed solutions.

As shown in Figure 7 (a), the hydrolysis mixtures of chitin in 12 M HCl at 25 °C show a clear reddish-brown precipitate after 14 h. This indicates that the reaction mixtures in 12 M HCl produce reductive sugar structures after 14 h even at 25 °C. However, Figure 7 (b) shows that a clear reddish-brown precipitate was observed after hydrolysis of cicada shells for 48 h in 12 M HCl at 25 °C.

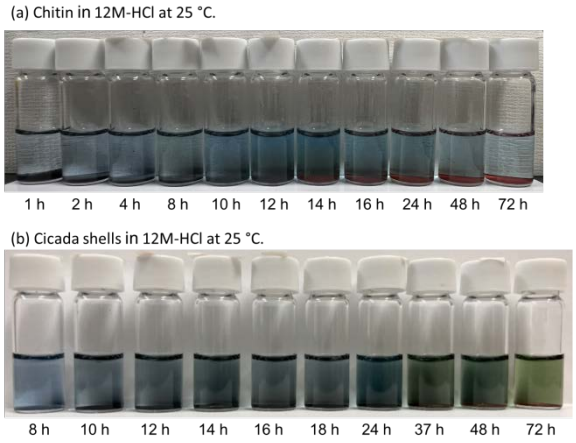

Figure 7. Benedict reaction of standard (a) chitin and (b) cicada shells hydrolyzed in 12 M HCl at 25 °C.

3.1.2. Detection of Amino Groups with Ninhydrin

Ninhydrin reaction with the mixtures of acid-hydrolyzed chitin and cicada shells in 6 M HCl at 110 °C are shown in Figure 8. For hydrolyzed chitin, the intensity of the spots from the ninhydrin reaction were all rather low; only for spots from the later stages of the reaction (>8 h) can a little purple color be seen. The color of the spot from the ninhydrin reaction of hydrolyzed cicada shells (Figure 8 (b)) is clear in all cases.

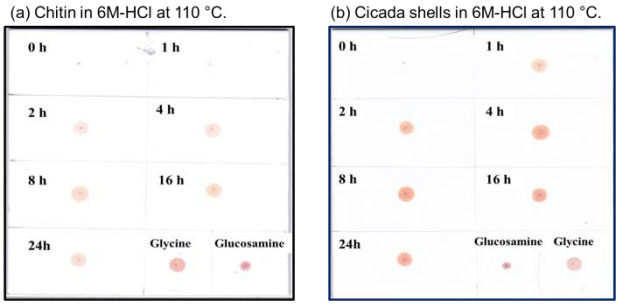

Figure 8. Ninhydrin reaction with the reaction mixtures of hydrolyzed chitin and cicada shells in 6 M HCl at 110 °C.

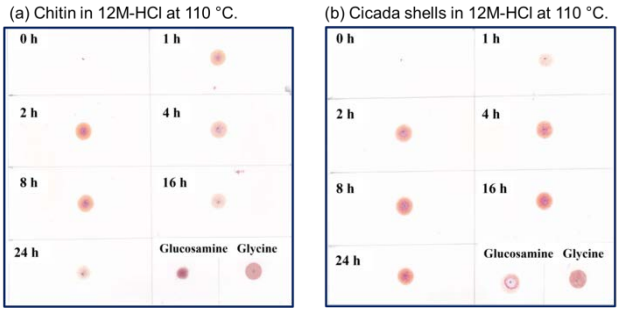

Figure 9. Ninhydrin reaction with the reaction mixtures of hydrolyzed chitin and cicada shells in 12 M HCl at 110 °C

Figure 9 shows the results of ninhydrin tests after acid hydrolysis of chitin and cicada shells in 12 M HCl at 110°C. A comparison of the ninhydrin responses of acid-hydrolyzed solutions at 110°C revealed that solutions obtained using 12 M HCl (Figure 8) gave clearer responses than those obtained using 6 M HCl (Figure 9). The spot color of ninhydrin reactions of acid-hydrolysis solution of chitin was less intense than that of cicada shells. Although the same mass of standard chitin and cicada was used for these acid-hydrolyses assays (10 mg), the cicada samples might contain protein contaminants. The ninhydrin reaction of solutions from amino acids obtained from proteins in the cicada shells.

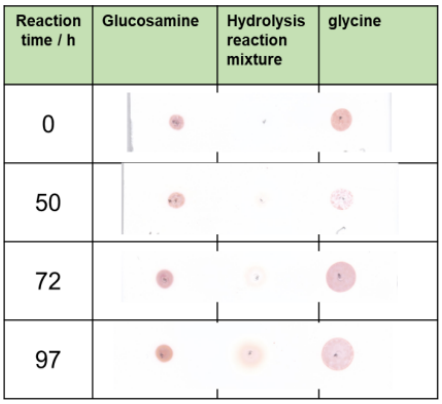

Figure 10. Ninhydrin reaction with reaction mixtures of hydrolyzed chitin in 12 M HCl at 25 °C

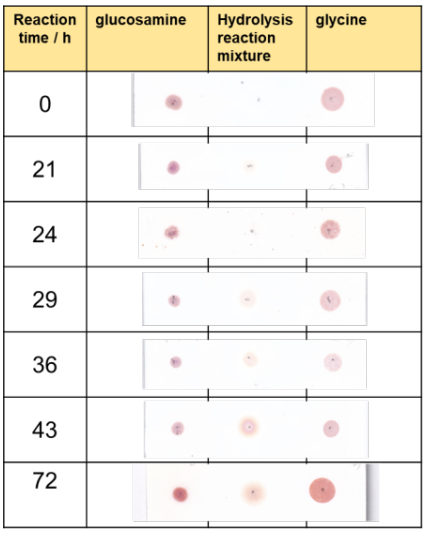

Figure 11. Ninhydrin reaction with reaction mixtures of hydrolyzed cicada shells in 12 M HCl at 25°C

Figures 10 and 11 show the results of hydrolysis in 12 M HCl at 25 °C. Ninhydrin reactions with mixtures of chitin hydrolyzed in 12 M HCl at 25 °C were positive after 72 h. These results reveal free amino groups in the glucosamine or chitosan structure upon hydrolysis in 12 M HCl at 25 °C. The ninhydrin reactions with the mixtures of hydrolyzed cicada shells were positive after 29 h. The positive ninhydrin reactions could also suggest the presence of free amino groups of amino acids as well as glucosamine or chitosan structure.

3.2. Dialysis of Acid-Hydrolyzed Cicada Shells and Gel Formation

The front parts (Figure 4) of the thorax and abdomen were cut out from the complete structure of a cicada shell. Dialysis was carried out in seven steps as follows:

(1) The total mass (100 mg) of the parts from 10 cicada shells was mixed with 12 M HCl (10 mL) in a 20 mL glass bottle. (2) After covering the bottle with a screw cap, the mixture was agitated several times and allowed to stand at 25 °C. (3) After 1 h, the bottle was agitated again. (4) The obtained upper solution (6.0 mL) was separated into three 2 mL aliquots. Each aliquot was added to a different molecular weight cutoff membrane tube (3,900, 8,000, and 12,000–14,000 Da) containing water (18 mL) to give a gel suspension. (5) The resulting suspensions were dialyzed against distilled water (2.0 L). (6) The outside distilled water was changed two times and the electrical conductivity of the suspension in the membrane tube was measured to be 5–10 μS/cm; the pH was 7.0. (7) The resulting gel suspensions in the membrane tubes were lyophilized to afford amorphous powders.

After the seven-step procedure, 12 M HCl (6.0 mL) was added to the resulting mixture of cicada shells and the procedure from steps (2) to (7) was carried out again. The obtained mass of lyophilized gel is summarized in Table 1.

The data presented in Table 1 show the relationship between the obtained weight (mg) of amorphous gel, the size of the molecular weight cutoff, and the reaction time of cicada shells in 12 M HCl at 25 °C. The amorphous gel was obtained by removing lower molecular-weight substances such as amino acids, minerals, halogens, sugars, and others. The total mass obtained by lyophilization was 48.3 mg, which corresponded to almost half the mass of the initial cicada shell parts (100 mg). An example of the resulting gels is shown in Figure 12.

Figure 12. An example of the gel generated from cicada shells after dialysis against 12,000-14,000 Da molecular weight cutoff.

Table 1.

Table 1. Chitin from molecular cutoff cicada shells dissolved in 12 M-HCl at 25 °C

	Yield (mg) of lyophilized chitin depending on Molecular cutoff		
Reaction time / h	3.5 kD	8 kD	12-14 kD
1	7.5	3.7	10.9
2	2.1	2.2	4.1
3	2.5	1.8	2.5
4	1.0	0	0
5	6.0	2.0	2.0

Chitin is soluble in 12 M HCl, but insoluble in neutral water, in which it forms an amorphous gel. The resulting mixture after dialysis does not include lower molecular-weight compounds; it is composed primarily of chitin.

3.3. Cicada and Environment Conception

Cicada adults on a tree lay eggs in the branches of the tree. Cicada hatchlings from the eggs go to the ground near the tree [1]. They live underground as nymphs for several years while they suck the sap from the xylem roots. Eventually, they climb up the stem of the tree and molt to become adults, who fly out of the tree leaving the cicada shells. The cyclic process repeats again and again.

There is also a chemical cycle that proceeds through cicada shells. Cicada exoskeleton and shells are mainly composed of chitin, which has a polymer structure constructed from the monomer *N*-acetyl-glucosamine. Glucosamine is made from glucose and amino acids. The metabolic route leading to the formation of chitin from glycogen [12] is shown in Figure 13 [13,14]. Nymphs feed on amino acids (glutamine) from xylem trees, use glucosamine in their body, and make *N*-acetylglucosamine, which they polymerize through uridine-diphospho-*N*-acetylglucosamine to chitin. Nymphs underground extract

xylem fluids, which include water, minerals, and amino acids [2] [12–16].

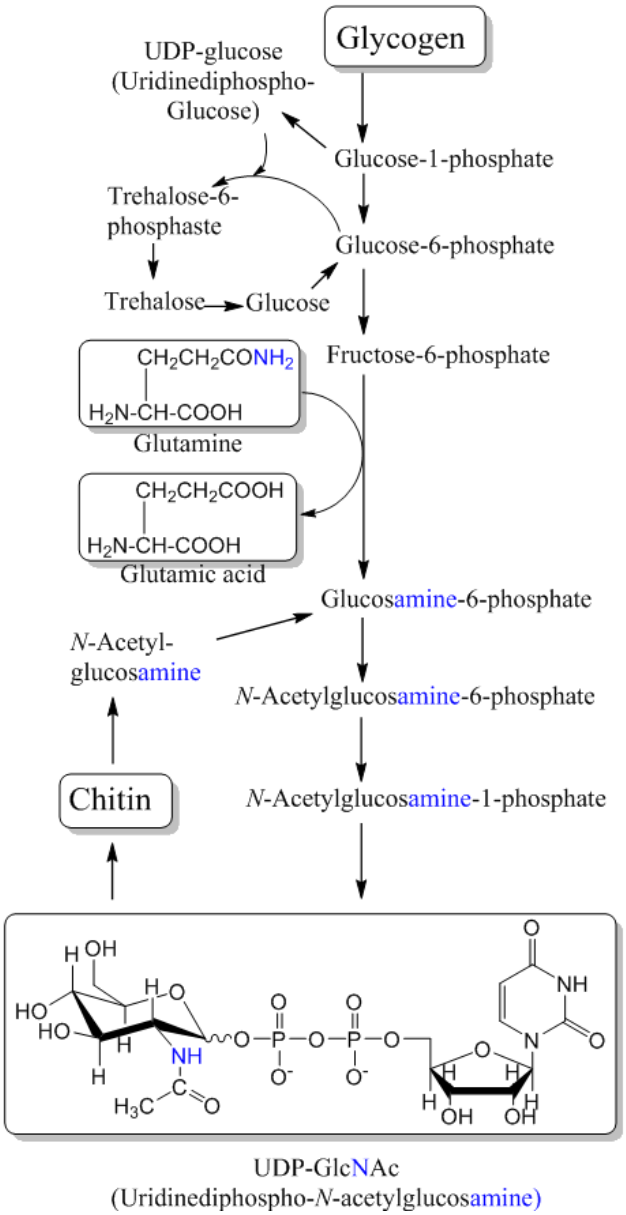

Figure 13. Metabolic pathway from glycogen and amino acid to chitin. Enzyme names are omitted from the metabolic paths and cycles.

Many kinds of amino acids, including glutamic acid and aspartic acid, are found in xylem roots (*C. canadensis*) after hydrolysis in 6 M HCl at 110°C [16]. However, because glutamic and aspartic acids are formed from the hydrolysis of glutamine and asparagine, respectively, the total amounts of glutamic and aspartic acids detected reflect the sum of the respective amino acids [16]. The amino acid arginine, which is not detected in xylem roots, was found in the gut of cicada. This suggested that arginine was produced in the gut of cicada by endosymbiotic bacteria [16]. For example, "Cicadas are dependent on the essential bacterial symbionts *Sulcia* and *Hodgkinia*" [1]. Such bacterial symbionts in cicadas have a genomic relationship with the fungal parasite, *Ophiocordyceps* [1]. These reports can provide useful teaching materials for studying cycles of chemicals in the environment.

There are many chitin materials with biological origins, such as clam shells, and the exoskeletons of crab, shrimp, and pill bug; however, these materials generate carbon dioxide in hydrochloric acid, while cicada shells do not. The difference in chemical reactions between biological materials can be applied to study environmental differences between these organisms.

This study provides methods to detect reducing sugars and amino groups from chitin that are suitable for practical use in the classroom. The protocols can be used as aids to discuss environmental chemical cycles.

4. Conclusions

(1) Cicada shells are mainly composed of chitin, which can be hydrolyzed to oligomers of *N*-acetylglucosamine and/or glucosamine. These chemical structures were detected as reducing sugars using the Benedict reagent and as amino groups using ninhydrin.

(2) The alternative procedures of using acid-mediated hydrolysis of standard chitin and cicada shells for teaching chemistry are proposed as follows: hydrolysis in 6 M HCl at 110 °C and 12 M HCl at 110 °C. These procedures may not be appropriate for all types of schools, whereas the milder reaction conditions, 12 M HCl at 25 °C for two or three days, will be suitable for practical use in most schools.

(3) When chitin solution from cicada shells in 12 M HCl at 25 °C was diluted in water, the resulting white gel can be used to demonstrate both the nature of gel materials and the very low solubility of chitin in water.

(4) Cicada shells can be used to highlight the relationship between cicadas and the environment. Cicada nymphs feed on amino acids and other nutritive substances from the xylem vessel. The connection between nutritive substances and chitin can be explored by observing the presence of reducing sugars and amino groups after the hydrolysis of chitin.

(5) Cicada shells are a widely available resource for teaching areas such as the chemicals in cicadas, the nature of gels, and the relationship between cicadas and the environment. Indeed, cicada shells constitute a versatile material for teaching many areas of science.

References

[1] Matsuura, Y., Moriyama, M., Łukasik, P., Vanderpool, D., Tanahashi, M., Meng, X.-Y., McCutcheon, J.P., Fukatsu, T. "Recurrent symbiont recruitment from fungal parasites in cicadas", *Proc. Natl. Acad. Sci. U. S. A.*, 115 (26), E5970–E5979, September 2019.

[2] Williams, K.S., Simon, C., "The ecology, behavior, and evolution of periodical cicadas", *Annu. Rev. Entomol.*, 40, 269–295, 1995.

[3] Maruyama, N., Terashima, Y., Munegumi, T., "Meaning and application using 'cicada shells' as teaching materials for observation of insect's structure: Practical lessons for teaching training education", *Journal of Japan Society of School Subject Content Education*, 7 (1), 17–28, March 2021.

[4] Donahue, C.J., Marini, A., Savu, C., Yehya, H., "Electroless deposition on three substrates: Brass washers, cicada exoskeletons, and beetles", *J. Chem. Ed.*, 96 (10), 2279–2285, July 2019.

[5] Hudson, R., Glaisher, S., Bishop, A., Katz, J.L., "From lobster shells to plastic objects: A bioplastics activity", *J. Chem. Ed.*, 92 (11), 1882–1885, August 2015.

[6] Luzzi, E., Ambrogi, V., Filippone, G., Liguori, B., de Luna, M.S., Aprea, P., "Increasing awareness of materials and the environment: Hands-on outreach activity presenting water purification materials and concepts", *J. Chem. Ed*., 98 (4), 1296–1301. March 2021.

[7] Kobayashi, S., Kiyosada, T., Shoda, S., "Synthesis of artificial chitin: Irreversible catalytic behavior of a glycosyl hydrolase through a transition state analogue substrate", *J. Am. Chem. Soc*., 118 (51), 13113–13114, December 1996.

[8] Tews, I., van Scheltinga, A.C.T., Perrakis, A., Wilson, K.S., Dijkstra, B.W., "Substrate-assisted catalysis unifies two families of chitinolytic enzymes", *J. Am. Chem. Soc*., 119 (34), 7954–7959, August 1997.

[9] Brameld, K.A., Goddard III, W.A., "The role of enzyme distortion in the sight displacement mechanism of family 19 chitinases", *Proc. Natl. Acad. Sci. U. S. A*., 95 (8), 4276–4281, April 1998.

[10] Ohno, T., Armand, S., Hata, T., Nikaidou, N., Henrissat, B., Mitsutomi, M., Watanabe, T., "A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT 6037", *J. Bacteriol*., 178 (17), 5065–5070, September 1996.

[11] Thakur, D., Bairwa, A., Dipta, B., Jhilta, P., Chauhan, A., "An overview of fungal chitinases and their potential applications", *Protoplasma*, 260 (4), 1031–1046, February 2023.

[12] Vardanis, A. "Glycogen synthesis in the insect fat body", *Biochim. Biophys. Acta*, 73 (4), 565–573, August 1963.

[13] Kramer, K.J., Koga, D., "Insect chitin: Physical state, synthesis, degradation and metabolic regulation", *Insect Biochem.*, 16 (6), 851–877, June 1986.

[14] Muthukrishnan, S., Merzendorfer, H., Arakane, Y., Kramer, K.J., "Chitin metabolism in insects", *Insect Mol. Biol. Biochem*., 193–235, 2012.

[15] Cheung, W.W.K., Marshall, A.T., "Water and ion regulation in cicadas in relation to xylem feeding", *J. Insect Physiol.*, 19 (9), 1801–1816, September 1973.

[16] Christensen, H., Fogel, M.L., "Feeding ecology and evidence for amino acid synthesis in the periodical cicada (*Magicicada*)", *J. Insect Physiol*., 57 (1), 211–219, January 2011.