Rice 'n Beans or Ricin Beans? A Deadly Swap

by
Ann T.S. Taylor
Department of Chemistry
Wabash College, Crawfordsville, IN

"Hi Mom, I'm home!" Taylor gave her mom a hug and handed her a laundry bag. "I'm starving. What's for dinner?"

"Rice and beans … that's all we can afford after this semester's tuition bill." Mom laughed.

"Better make sure they aren't *ricin* beans, dear." Dad peeked his head over the newspaper. "But seriously, Taylor, I'm so glad you're home! How were midterms?"

Taylor joined her dad at the breakfast nook table. "Good, though I'm not done yet. I have a paper assignment due next Friday—I have to find a natural poison and explain how it works. What was up with the ricin beans?"

Dad chuckled and showed Taylor an article. "Remember the poisonous letter sent to a politician? When they confronted one of the suspects, he thought they were talking about rice, not ricin.[1] But then again, he was an Elvis impersonator, he probably doesn't read *National Geographic* very much."

Mom chimed in. "You mean that *National Geographic* article about how ricin works?[2] I thought it was fascinating that ricin is a protein found in castor beans. I guess that's another reason to avoid Grandma's castor oil fix for everything."

Taylor was quiet for a minute. "Ricin comes from castor beans? That might be perfect for my project. Is that the poison that was on *Breaking Bad* that one time?[3] It's strange to think that a protein from a bean plant could kill a person. How does the plant not die from it?"

"That does sound perfect for your project, Taylor," Dad replied. "And remember, there are lots of poisonous things in nature. Natural doesn't mean not dangerous—just think about hemlock, mushrooms, and puffer fish."[4]

"Taylor, when you are done with your project, could you please email Dad and I something we could understand?" Mom asked as she joined them at the table. "I'd like to know more about how ricin affects cells, but that is definitely your area of expertise."

"Sure," Taylor replied. "I appreciate how supportive you are of my education. But can we talk about something else during dinner? Poison and dinner just don't go well together."

"Definitely. I don't want to think about ricin beans as I eat my rice and beans either," Dad quipped. "So how is your dating life? No, I take it back…that makes me almost as sick to my stomach as poison discussion does!"

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1 *ABC News* video story. April 24, 2013. Ricin suspect released: "I don't even eat rice"; remark occurs at 0:55 in video at http://abcnews.go.com/GMA/video/ricin-letters-mailed-obama-elvis-impersonator-suspect-released-19029161, accessed 6/2/14.

2 Newman, C. April 19, 2013. Ricin back in the headlines: what is it? *National Geographic News*, http://news.nationalgeographic.com/news/2013/13/130417-ricin-letter-poison-obama-roger-wicker-toxicology/, accessed 5/27/15.

3 Locker, M. April 17, 2013. Ricin attack sadly familiar to fans of *Breaking Bad. Time.* http://entertainment.time.com/2013/04/17/ricin-attacks-sadly-familiar-to-fans-of-breaking-bad/ accessed 5/27/15.

4 *Illinois Poison Center Blog.* September 16, 2011. Mother Nature's most dangerous toxins. http://ipcblog.org/2011/09/06/mother-natures-most-toxic/ accessed 5/27/15.

Part I – An Early Experiment

Taylor comes to you and the rest of your small group, asking for help with this project. You start digging in the literature and find some useful references.

An early experiment into the mechanism of ricin was published in 1971 (Lin et al.). The experiment measured the incorporation rate of ^{3}H labelled leucine, ^{3}H labelled thymidine, and ^{3}H labeled uridine into tumor cells treated with varying concentrations of ricin (Figure 1).

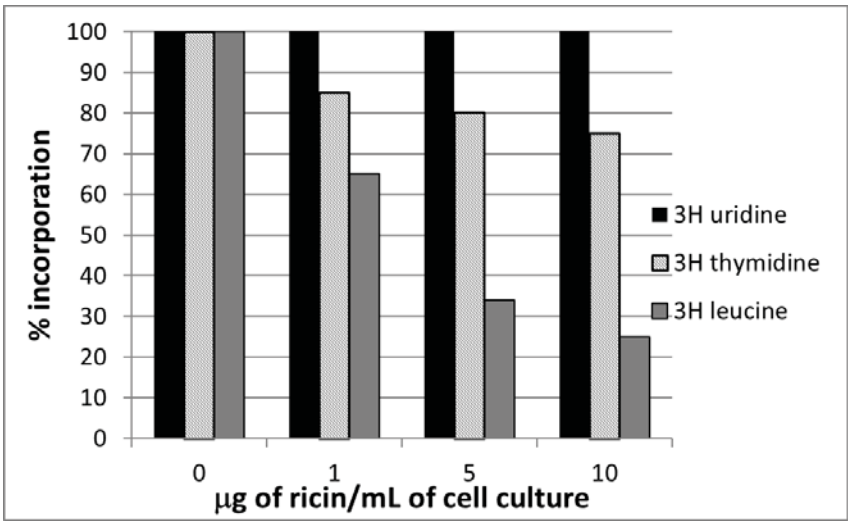


Figure 1. Incorporation of tritiated reagents in tumor cells (Lin et al., 1971).

Questions

1. Why were these three particular radioactive tracers used in this experiment? What process was each tracer monitoring?

2. What do you think was the hypothesis the researchers were testing?

3. What new hypothesis can be formed from these results?

4. Design an experiment to test your hypothesis.

Part II – Inhibition In Vitro

Another study looked at ricin inhibition of in vitro translation (Olsnes and Pihl, 972).

5. Make a list of all the components you would need to make an in vitro translation system.

6. In this experiment, the researchers "cheated"—they used a cell extract from rabbit reticulocytes. Mark with an asterisk all the components you listed in (5) that would be provided in a cell extract.

7. The researchers also added 15 mM creatine phosphate and 50 µg/mL creatine kinase. What is the role of these components?

8. Look at the figure legend for Figure 2.
   a. Why was the system pre-incubated for 40 minutes in the absence of labelled compounds?

   b. What is the role of the poly-U?

   c. What process did this experiment monitor?

   d. Why is the background level of incorporation not zero?

9. What conclusion can be drawn from this experiment?

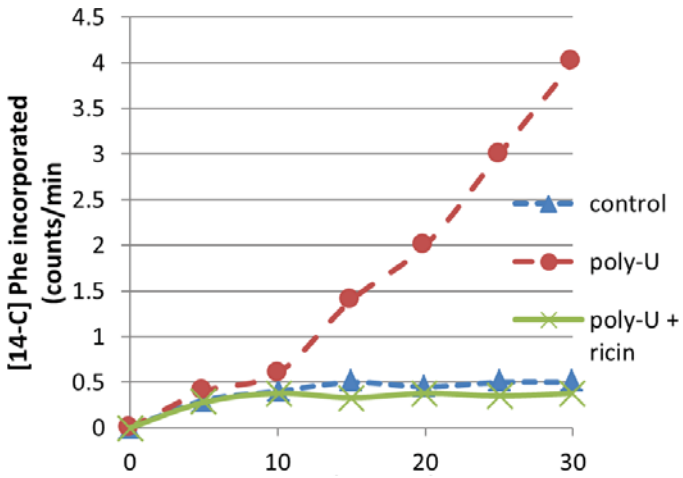
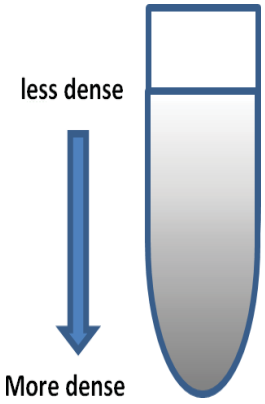


Figure 2. Effect of ricin on polyphenylalanine synthesis. A cell free system was preincubated at 28° for 40 minutes in the absence of labelled compounds. Then 4 µCi of ^{14}C phenylalanine was added, and the system was divided into three equal samples. One sample (▲) was used as a control, another sample (●) contained 100 µg of poly-U and a third sample (×) contained 100 µg of poly-U and 0.5 µg of ricin. The samples were incubated at 28°C and aliquots were removed as indicated, and the acid-precipitatable radioactivity was determined. *Source:* Redrawn from Figure 2 of Olsnes and Phl, 1972.

Part III – Ribosomes

10. In the next experiment, the researchers used sucrose gradients to isolate mRNA's with differing amounts of ribosomes attached. For each trial, RNA is isolated from cells and layered on a gradient of sucrose solutions, which provides a density gradient.
    a. Ribosome fragments are designated by their "sedimentation coefficient"; the more dense the unit, the farther it travels towards the bottom of the tube in density gradient centrifugation. In the centrifuge tube shown below, predict the relative position of the 40S subunit, the 60S subunit, the 80S complete ribosome, free mRNA, mRNA bound to an intact ribosome at the beginning of translation, mRNA that is almost done being translated, an mRNA bound to several ribosomes at various stages of translation, free amino acids, and a polypeptide chain that has been released from a ribosome.



A cell free system was incubated with ^{14}C leucine with ribosomes in a cell free extract, then fractionated via centrifugation on sucrose gradients. A hole was poked in the bottom of the tube and fractions were collected; the absorbance at 260 nm (a measure of the nucleotide content; left axis, ●) and radioactivity (right axis, ×) was measured in each fraction. For a control, the ribosomes were treated with a compound abbreviated ATA, which prevents initiation of translation.

    b. Why is most of the radioactivity in the lighter fractions?

    c. The 80S complex is indicated in all the frames. What does the "hump" in the fractions closer to the bottom of the tube reveal?

    d. Compare the ATA only and ricin only panels.

    e. What is the significance of the ATA + ricin data?

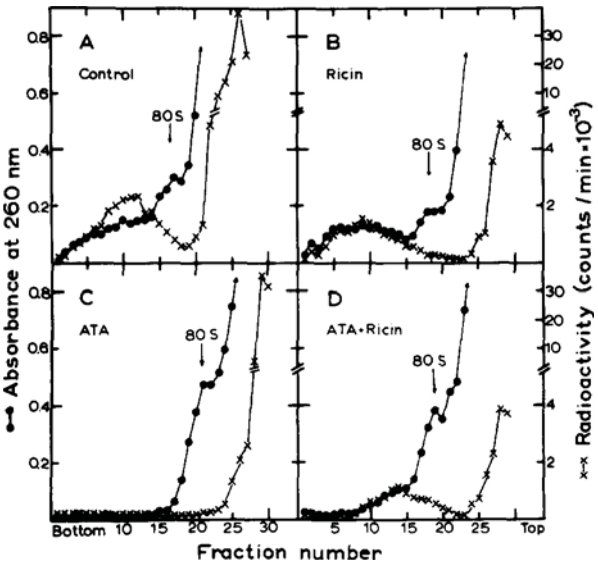


Figure 3. Ribosome sucrose gradients upon treatment with ricin or a translation inhibitor, ATA. *Source:* Olsnes and Pihl, 1972.

Part IV – What Does Ricin Affect?

Another question might be, does ricin directly affect the ribosome, or does it affect another component of translation? To answer this question, another team of researchers also used an in vitro translation system (Sperti et al., 1973), except they used purified ribosome components and elongation factors (see Figure 4).

11. Which subunit's functioning is affected by ricin treatment? Explain how controls are used in this experiment.

Subunit		*Radioactivity incorporated (d.p.m.)*
40S	*60S*	
Control	—	26
—	Control	146
Control	Control	3199
Ricin-treated	—	27
—	Ricin-treated	37
Ricin-treated	Ricin-treated	228
Control	Ricin-treated	137
Ricin-treated	Control	2916

Figure 4. "Poly(U)-directedpolyphenylalanine synthesis catalysed by isolated control subunits and by isolated subunits treated with ricin. Isolated subunits incubated in the presence and in the absence of ricin were sedimented through discontinuous sucrose gradients and tested for polyphenylalanine synthesis....The results are means of two experiments with 0.41 E_{260} unit of control 40S, 1.26 E_{260} units of control 60S, 0.38 E_{260} unit of ricin-treated 40S and 1.18 E_{260} units of ricin-treated 60S subunits." *Source:* Table 2 and caption in Sperti et al., 1973.

About a decade later, another team of researchers treated ribosomes with ricin, then looked at migration of the RNA subunits of the ribosome in gels (Endo et al., 1987). They observed that the 28S subunit, a component of the 60S subunit migrated differently, and sequenced it by radioactively labeling the RNA, then used ribonucleases with different sequence specificities to hydrolyze the RNA, and ran the samples on a gel (Figure 5). The odd lanes are control samples, while the even lanes have been treated with ricin.

12. In lanes 7 and 8, the 28S RNA was treated with enzymes that cleave after A or U. The sequence of bases 4319–4327 is shown to the left of the gel; the zig-zagging is for typographical reasons. In lanes 11 and 12, the samples were held at high pH, which preferentially cleaves sites that are apurinic or apyrimidinic (lack the base of the nucleotide). Based upon the sequencing gel to the right, determine how ricin alters rRNA.

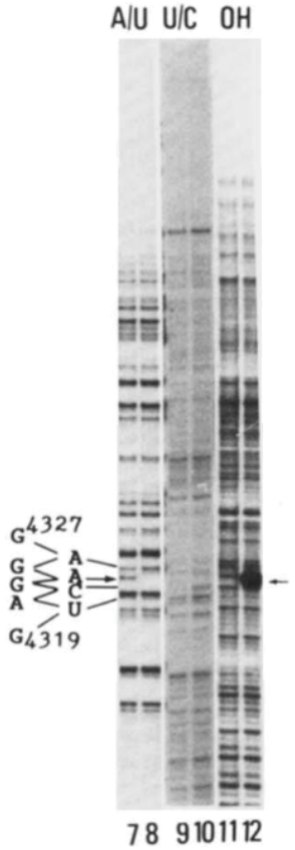


Figure 5. Poly(U)-directedpolyphenylalanine synthesis catalysed by isolated control subunits and by isolated subunits treated with ricin. *Source:* Detail of Panel A, Figure 3, in Endo et al., 1987.

13. Brainstorm at least three ways this change could alter ribosome function. For one of your ideas, propose an experiment to test your hypothesis.

Activity: Divide your group into two subgroups that each selects a different "Choose Your Own Experiment" assignment. Each subgroup should then combine with another subgroup that has chosen the same asignment. After answering your questions, you will reform your original groups and share what you have learned, and then individually write a one page summary of the mechanism of ricin toxicity that is appropriate to share with Taylor's parents.

Choose Your Own Experiment #1: EF Interactions with Ribosome

The site of depurination is in the middle of a highly conserved loop in the 28S rRNA, conveniently called the Sarcin-Ricin Loop (SRL), which is far from the active site. Elongation factor EF-1 associates with post-translocation ribosomes (peptidyl-tRNA in the P site), while EF-2 binds only to pre-translocation ribosomes (peptidyl-tRNA in the A site). To evaluate the effect of ricin upon elongation factor binding, Nilsson and Nygard (1986) incubated reticulocyte lysates with radioactively labeled EF-1 or EF-2, then isolated the ribosomes and measured the amount of EF-1 and EF-2 present.

1. As a reminder, describe the role of EF1 and EF2 in translation. (*Hint:* these are eukaryotic factors.)

2. Which EF is more affected by ricin treatment?

3. What are two possible explanations for reduced EF-X (either 1 or 2, based on how you answered the preceding question) binding affinity?

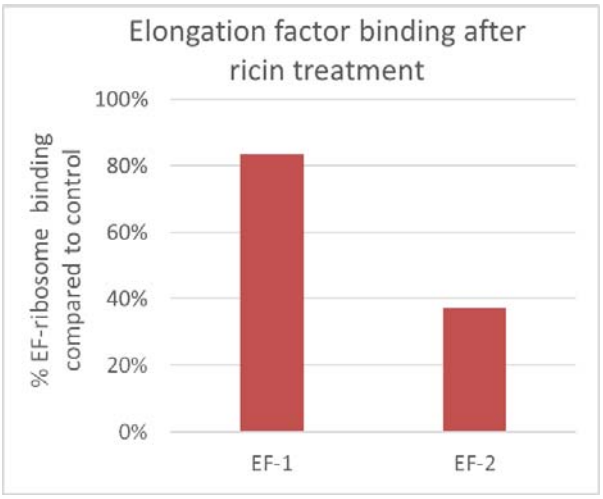


Figure 6. Binding of radioactively labelled elongation factors to ribosomes. Based on Nilsson and Nygard, 1986.

4. To evaluate these hypotheses, γ ^{32}P-GTP was incubated with ribosomes and EF-1, and the rate of ^{32}P-PO_4 release was measured. Which hypothesis does this data support? Why?

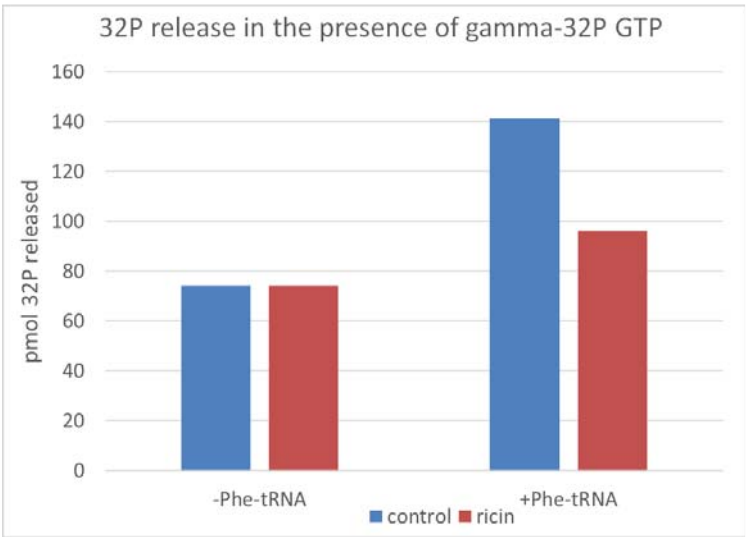


Figure 7. Release of $^{32}PO_4^{3-}$ from ribosomes treated with γ ^{32}P-GTP and EF-1 in the presence and absence of Phe-tRNA. Based on Nilsson and Nygard, 1986.

Choose Your Own Experiment #2: What About Ricin Itself?

1. View the structural features of ricin and its interactions with the ribosome at:
   http://www.proteopedia.org/wiki/index.php/Ricin
   Describe the interactions between ricin and the ribosome.

2. The only documented case of ricin killing someone was via injection. There is also concern about "weaponized" ricin, which can be inhaled. Why would a protein poison be more lethal via injection or inhalation than by drinking or eating?

3. Say ricin is injected into a person. Describe the movement of the protein that must occur for ricin to encounter a ribosome.

4. Ricin is a heterodimer connected with a disulfide bond. One subunit contains a galactose binding domain; the other contains the glycosylase activity. If the disulfide bond is broken before ricin enters the cell, it has no effect upon the ribosome. Explain.

5. Ricin is not toxic to the developing castor bean plant because it is located in the "protein body," a seed organelle analogous to a vacuole, and is hydrolyzed a couple of days after germination. Suggest how ricin is synthesized so that it isn't toxic to the ribosome that is making it.

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