NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Skeleton in the Closet:
Age Estimation Using the D/L Asp Ratio

by

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Part I – The Unexpected Discovery

"Never a dull moment in this job, huh?" You look up as your supervisor drops a new case file on your desk. As a seasoned forensic chemist in the New York State Police Forensic Investigation Center, you had to agree.

Your supervisor fills you in on the rough details of the case. Apparently, a young couple was excited to buy their first home in a community outside Albany. The previous owner of the house was an elderly gentleman who had moved into a retirement home and later passed away. His estranged children did their best to clean out the house, but they were mostly anxious to just be rid of the property. Shortly after moving into the house, the young couple realized that many things had been left in the attic. One day while removing these items, they opened a closet in the attic and, to their horror, found an almost complete skeleton inside. At first, they thought it was an old Halloween decoration, but on closer inspection, it looked too real. They called the police who confirmed that it was indeed a well-preserved female human skeleton.

"Our DNA team has already extracted and profiled the skeleton's DNA, but there were no hits in the DNA databases. We need more to go on, so I'd like for you to see if you can get an estimate of how old the victim was when they died. That could help us see if there are any missing person's reports that match," your supervisor tells you. "I'll need your report as soon as you are finished."

You decide to determine the age of the victim when they died using a method that measures aspartic acid racemization in teeth. Most naturally occurring proteins are made from L-amino acids, as opposed to their enantiomers, the D-amino acids. The structures of L and D aspartic acid can be seen in Figure 1. Instead of the more common R and S configurations used in organic chemistry, the amino acids are termed L and D based on if the stereochemistry at the α carbon is the same configuration as L or D glyceraldehyde.

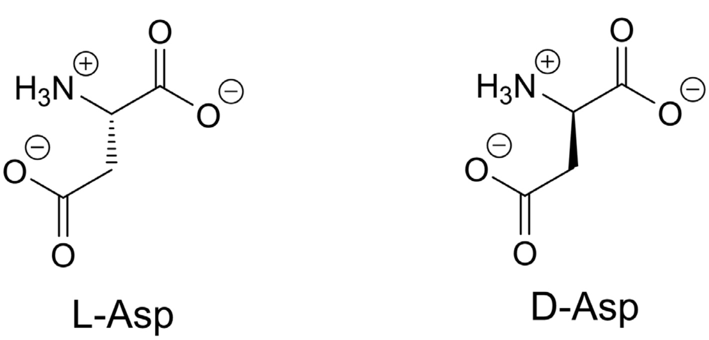

Figure 1. The structures of L and D aspartic acid.

The natural process of racemization slowly converts the L form to the D form until a 50/50 racemic mixture results. The racemization process is very slow, with half-lives on the order of hundreds to thousands of years. Therefore, D-amino acids are usually not detectable in proteins that are rapidly turned over in the body and are only detectable in structural proteins that are made early in life and then not turned over, such as in enamel and dentin. Dentin, the part of the tooth beneath the enamel (Figure 2), can be analyzed for this D:L ratio and provide an estimate of age.

Premolar tooth samples were decontaminated, followed by root dissection to obtain dentin. Aliquots of dentin samples were treated with 6 M HCl at 100 °C for 6 hours to degrade the proteins to their constituent amino acids. Total amino acid analysis was conducted by gas chromatography (GC) using D-Met as an internal standard.

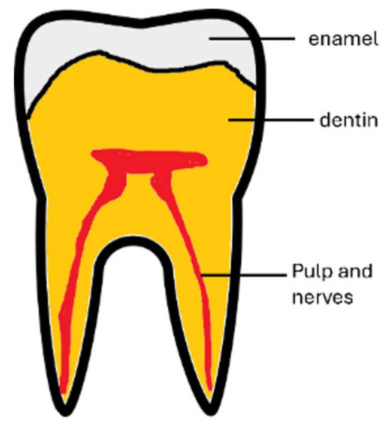

Figure 2. Tooth structure.

Questions

1. Why is it important to use an internal standard for this application?

2. What features of D-Met make it good for use as an internal standard in this application?

3. Provide a brief overview of the kinds of experiments you need to run on the GC and the data you need to collect.

Part II – Determining Biological Age

You first make samples of the standards containing differing concentrations of D-Asp and L-Asp ranging from 0.25 to 2.00 mg/mL with a constant concentration of 0.2 mg/mL of D-Met as the internal standard. You run these samples on the gas chromatograph and integrate the resulting peaks to provide peak areas.

You also require the D:L Asp ratio in dentin of different ages, so you determine this ratio using gas chromatography and donated premolars of known age. Finally, you collect data from the dentin in the skeletal remains.

After running the experiments, you obtain the data in Tables 1–3.

Table 1. Standards of D-Met, D-Asp and L-Asp and their peak areas from GC.

[D-Met] mg/mL	*[L-Asp] mg/mL*	*[D-Asp] mg/mL*	*L-Asp peak area*	*D-Asp peak area*	*D-Met peak area*
0.2	0.25	0.25	4234	4259	12521
0.2	0.50	0.50	8444	8310	13113
0.2	0.75	0.75	11864	11889	12492
0.2	1.00	1.00	15463	15390	12316
0.2	1.25	1.25	20747	19617	12564
0.2	1.50	1.50	24277	23651	12342
0.2	2.00	2.00	29810	30072	12639

Table 2. D-Asp ratios from dentin in various aged samples.

Dentin age (years)	*[D-Asp]/[L-Asp]*
5	0.057
8	0.061
12	0.063
20	0.070
25	0.074
40	0.087
45	0.091
60	0.104
75	0.116

Table 3. Data from the skeletal remains.

Trial	*L-Asp peak area*	*D-Asp peak area*	*D-Met peak area*
1	11766	1629	12643
2	11831	1636	12972
3	11655	1619	12678

Questions

1. Use the information above to determine the most likely age of the victim when they died.

2. Write a brief report of your findings for your supervisor and how you came to them. Include the data given and numbers calculated in tables and any graphs you prepare (properly labeled with a number, caption, and referred to in the text).