## Activity Eight: Amino Acid Sequences and Evolutionary Relationships

Proteins play a major role in living organisms. Amino acids are the building blocks of proteins. The central dogma in molecular biology is that DNA determines the sequence of amino acids.

Biologists have perfected techniques for determining the sequence of amino acids in proteins. By comparing the amino acid sequences in homologous proteins of similar organisms and of diverse organisms, evolutionary relationships that might otherwise go undetected can be determined. Biologists believe that the greater the similarity between the amino acid sequences of the two organisms, the closer their relationship. Biologists have found that such biochemical evidence compares favorably with other lines of evidence for evolutionary relationships.

In this investigation, you will compare amino acid sequences in proteins of several vertebrates. You will also study amino acid differences and infer evolutionary relationship among some diverse organisms.

**Part A. Comparing Amino Acid Sequences**

1. Hemoglobin, a protein composed of several long chains of amino acids, is the oxygen-carrying molecule in red blood cells. Examine figure 1, which compares corresponding portions of hemoglobin molecules in humans and five other vertebrate animals. The sequence shown is only a portion of a chain made up of 146 amino acids. The numbers on Figure 1 indicate the position of a particular amino acid in the chain. Human hemoglobin is being used as the standard for comparison.

Figure 1 Comparison of hemoglobin molecules

| | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Human** | THR | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| **Chimpanzee** | THR | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| **Gorilla** | THR | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| **Rhesus Monkey** | GLN | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| **Horse** | ALA | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| **Kangaroo** | LYS | LEU | SER | GLU | LEU | HIS | CYS | ASP | LYS | LEU | HIS | VAL | ASP | PRO | GLU |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 |
| **Human** | ASN | PHE | ARG | LUE | LUE | GYL | ASN | VAL | LEU | VAL | CYS | VAL | LUE | ALA | HIS |
| **Chimpanzee** | ASN | PHE | ARG | LUE | LUE | GYL | ASN | VAL | LEU | VAL | CYS | VAL | LUE | ALA | HIS |
| **Gorilla** | ASN | PHE | LYS | LUE | LUE | GYL | ASN | VAL | LEU | VAL | CYS | VAL | LUE | ALA | HIS |
| **Rhesus Monkey** | ASN | PHE | LYS | LUE | LUE | GYL | ASN | VAL | LEU | VAL | CYS | VAL | LUE | ALA | HIS |
| **Horse** | ASN | PHE | ARG | LUE | LUE | GYL | ASN | VAL | LEU | ALA | LEU | VAL | VAL | ALA | ARG |
| **Kangaroo** | ASN | PHE | LYS | LUE | LUE | GYL | ASN | ILE | ILE | VAL | ILE | CYS | LEU | ALA | GLU |

2. Construct a data table which indicates the number of amino acid differences in the hemoglobin of each organism in comparison with that of the human and the positions in which they vary. The headings in the table should read "Organisms", "Number of Amino Acid Differences", and the "Positions in Which They Vary".

**Part B. Inferring Evolutionary Relationships from Differences in Amino Acid Sequences**

1. Another commonly studied protein is cytochrome c. This protein, consisting of 104 amino acids, is located in the mitochondria of cells. There it functions a respiratory enzyme. Examine Figure 2. Using human cytochrome c as a standard, the amino acid differences between humans and a number of other organisms are shown.

**Figure 2**

| Species Pairings | Number of Differences |
|---|---|
| Human-chimpanzee | 0 |
| Human-fruit fly | 29 |
| Human-horse | 12 |
| Human-pigeon | 12 |
| Human-rattlesnake | 14 |
| Human-red bread mold | 48 |
| Human-rhesus monkey | 1 |
| Human-screwworm fly | 27 |
| Human-snapping turtle | 15 |
| Human-tuna | 21 |
| Human-wheat | 43 |

2. Use Figure 2 to construct a bar graph to show the amino acid differences between humans and other organisms.
3. Examine Figure 3. In this figure the cytochrome c of a fruit fly is used as a standard in comparing amino acid differences among several organisms. Construct a bar graph on Graph 2 to show these differences.

**Figure 3: cytochrome c species pairing**

| Species Pairings | Number of Differences |
|---|---|
| Fruit Fly-dogfish shark | 26 |
| Fruit Fly-pigeon | 25 |
| Fruit Fly-screwworm fly | 2 |
| Fruit Fly-silkworm moth | 15 |
| Fruit Fly-tobacco hornworm moth | 14 |
| Fruit Fly-wheat | 47 |

Part C: Analysis and Conclusion

1. Examine your data table from part A. On the basis of the hemoglobin similarity, what organisms appear to be most closely related to humans? Least closely related? Explain your answer.

   For #2-7 consult bar graphs from part B.

2. On the basis of differences in their cytochrome c, which organisms appear to be most closely related to humans?
3. Which organisms appear to be least closely related to humans?
4. Which pair of the following organisms appears to be least closely related to each other? (Snapping turtle-tuna, snapping turtle-rattlesnake, or snapping turtle-pigeon) Give a reason for your answer.
5. Agree or disagree with the following statement and give reasons to support your answer. "Fruit flies appear to be more closely related to silkworm moths than they are to screwworm flies."
6. Name the pair of organisms that appear to be equally related to humans but not equally related to each other. Explain you answer.
7. Is it possible that the organisms in question 6 could be equally related to humans but not equally related to each other? Explain your answer.

Part D: Critical Thinking and Applications

1. There is a difference of only one amino acid in one chain of the hemoglobin of humans and gorillas. What might have caused this difference?
2. If the amino acid sequences in the proteins of two organisms are similar, why will their DNA also be similar?
3. With more recent technology in biology, what organic molecule is probably the most valuable for study to determine evolutionary relationships? Explain your answer.