Name _____

Date _____ Class _____

C19 Analyzing Amino-Acid Sequences to Determine Evolutionary Relationships

Skills

- comparing and contrasting sequences of amino acids
- interpreting data

Objectives

- *Identify* the differences in the amino-acid sequences of the cytochrome c and hemoglobin molecules of several species.
- *Infer* the evolutionary relationships among several species by comparing amino-acid sequences of the same protein in different organisms.

PREPARATION NOTES

Time Required: 1 class period

Materials

- paper
- pencil

Purpose

No outside preparation is required for this lab.

You are a zoologist who specializes in the classification of vertebrates according to their evolutionary relationships. In your research, you examine the amino-acid sequences of particular protein molecules found in vertebrates to determine the degree of biochemical similarity between vertebrate species. Today you will compare portions of human cytochrome c and hemoglobin molecules with the same portions of those molecules in other vertebrate species. Your goals are to determine the differences in the amino-acid sequences of the molecules and to deduce the evolutionary relationships among the species.

Background

The biochemical comparison of proteins is a technique used to determine evolutionary relationships among organisms. Proteins consist of chains of amino acids. The sequence, or order, of the amino acids in a protein determines the type and nature of the protein. In turn, the sequence of amino acids in a protein is determined by the sequence of nucleotides in a gene. A change in the DNA nucleotide sequence (mutation) of a gene that codes for a protein may result in a change in the amino-acid sequence of the protein.

Biochemical evidence of evolution compares favorably with structural evidence of evolution. Even organisms that appear to have few physical similarities may have similar sequences of amino acids in their proteins and be closely related through evolution. Researchers believe that the greater the similarity in the amino-acid sequences of two organisms, the more closely related they are in an evolutionary sense. Conversely, the greater the time that organisms have been diverging from a common ancestor, the greater the differences that can be expected in the amino-acid sequences of their proteins.

Two proteins are commonly studied in attempting to deduce evolutionary relationships from differences in amino-acid sequences. One is cytochrome c, and the other is hemoglobin. **Cytochrome c** is a protein used in cellular respiration and found in the mitochondria of many organisms. **Hemoglobin** is the oxygen-carrying molecule found in red blood cells.

Procedure

Part 1—Cytochrome c

1. A cytochrome c molecule consists of a chain of 104 amino acids. The chart below shows the amino-acid sequence in corresponding parts of the cytochrome c molecules of nine vertebrates. The numbers along the side of the chart refer to the position of these sequences in the chain. The letters identify the specific amino acids in the chain.

Cytochrome c Amino-Acid Sequences

AA #	Horse	Chicken	Tuna	Frog	Human	Shark	Turtle	Monkey	Rabbit
42	Q	Q	Q	Q	Q	Q	Q	Q	Q
43	A	A	A	A	A	A	A	A	A
44	P	E	E	A	P	Q	E	P	V
46	F	F	Y	F	Y	F	F	Y	F
47	T	S	S	S	S	S	S	S	S
49	T	T	T	T	T	T	T	T	T
50	D	D	D	D	A	D	E	A	D
53	K	K	K	K	K	K	K	K	K
54	N	N	S	N	N	S	N	N	N
55	K	K	K	K	K	K	K	K	K
56	G	G	G	G	G	G	G	G	G
57	I	I	I	I	I	I	I	I	I
58	T	T	V	T	I	T	T	T	T
60	K	G	N	G	G	Q	G	G	G
61	E	E	N	E	E	Q	E	E	E
62	E	D	D	D	D	E	E	D	D
63	T	T	T	T	T	T	T	T	T
64	L	L	L	L	L	L	L	L	L
65	M	M	M	M	M	R	M	M	M
66	E	E	E	E	E	I	E	E	E
100	K	D	S	S	K	K	D	K	K
101	A	A	A	A	A	T	A	A	A
102	T	T	T	C	T	A	T	T	T
103	N	S	S	S	N	A	S	N	N
104	E	K	—	K	E	S	K	E	E

2. On a piece of scratch paper, write the name of each vertebrate in the chart on the previous page. Compare the amino-acid sequence of human cytochrome c with that of each of the other eight vertebrates. For each vertebrate's sequence, count the number of amino acids that differ from those in the human sequence. Write the number of differences in the amino-acid sequences under the vertebrate's name. When you have completed your comparisons, transfer your data to the data table below. As you do, list the eight vertebrates in order from fewest differences to most differences.

Cytochrome c Amino-Acid Sequence Differences Between Humans and Other Vertebrate Species

Species	Number of differences from human cytochrome c
monkey	1
rabbit	4
horse	6
chicken	7
turtle	8
frog	8
tuna	9
shark	14

♦ According to this line of evidence, which organism is most closely related to humans? Which is least closely related to humans?

The chimpanzee is the most closely related to humans. The shark is the least closely related.

♦ Frog and turtle cytochrome c molecules have the same number of differences from human cytochrome c. Which vertebrate, frog or turtle, would you put higher on the list? Explain.

Answers may vary. Because reptiles are more advanced than amphibians, students should list the turtle ahead of the frog.

Part 2—Hemoglobin

3. Look at the amino-acid sequences shown below. These sequences are portions of the hemoglobin molecules of five organisms. The portion of the chains shown are from amino acid number 87 to amino acid number 116 in a sequence of 146 amino acids.

Hemoglobin Amino-Acid Sequences

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

4. Compare the amino-acid sequence of human hemoglobin molecules with that of each of the other four vertebrates. For each vertebrate's sequence, count the number of amino acids that differ from the human sequence and list them in the table below. Be sure to list the animal species in descending order according to their degree of evolutionary closeness to humans.

Hemoglobin Amino-Acid Sequence Similarities Between Humans and Other Vertebrate Species

Species	Number of differences from human hemoglobin
chimpanzee	0
gorilla	1
monkey	2
horse	5

◆ In the study of hemoglobin, which vertebrate is most closely related to humans? Least closely related?

The chimpanzee is the most closely related; the horse is the least closely related.

Analysis

5. What are some methods biologists use to determine evolutionary relationships?

Methods include biochemical comparison, examination of the fossil record, and evaluation of existing and vestigial structures in modern organisms.

6. Why can it be said that proteins behave like molecular clocks?

Proteins can be said to behave like clocks because they change gradually over time due to mutations. The number of differences in amino-acid sequences might be considered a measure of the passage of time.

7. When the portions of the gorilla and human hemoglobin molecules were compared, there was only one difference in the amino-acid sequence. What could have been responsible for this change?

a mutation in the DNA of one species or the other after the two lines diverged

8. If the amino-acid sequences are similar in gorillas and humans, will the nucleotide sequence of their DNA also be similar? Why or why not?

Yes, because the amino-acid sequence of a protein is encoded by the nucleotide sequence of DNA

Conclusions

9. Examine the data table you completed in step 2 of Part 1. The values listed for the chicken and the horse differ by only one. Can you deduce from this that the chicken and the horse are closely related to each other? Why or why not?

No. The data in the table resulted from comparing chickens and horses with humans, not with each other.

10. How is biochemical comparison different from other methods of determining evolutionary relationships?

Biochemical comparison is based directly or indirectly on the nucleotide sequence of DNA, which determines the structure and appearance of an organism. Biochemical comparison enables biologists to collect numerical data that shows the degree of similarity between the DNA of different organisms. It can also be used to infer the passage of time. Other methods rely on more indirect comparisons, such as comparisons of structure and appearance, or on determinations of age based on relative or absolute dating of fossils.

Extensions

11. Do library or on-line research to discover what other types of molecules can be used to determine the evolutionary relationships among organisms based on biochemical comparisons.

12. Do research to find out how biologists determine the amino-acid sequence of a protein molecule.