

Analysis of Hemoglobin Variants

Routine electrophoretic surveys of human blood samples have revealed hundreds of variant hemoglobins. Most are rare and differ from normal hemoglobin by a single amino acid substitution. Some like HbS (Sickle-cell hemoglobin) produce severe clinical problems; others produce no ill effects. This problem set will deal with two different hemoglobin variants which affect the β -chain. Hemoglobin-G Couthatta was reported in two groups of Native Americans and is harmless. Hemoglobin Southampton was discovered in England and causes severe hemolytic anemia. Each variant has been purified and its sequence determined. The 146 amino acid sequence for the normal human hemoglobin β -chain is given below for reference in the questions that follow.

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1           10           20           30
NH3-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-Ser-Ala-Val-Thr-Ala-Leu-Trp-Gly-Lys-Val-Asn-Val-Asp-Glu-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-
      40           50           60
Leu-Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys-Val-
      70           80           90
Lys-Ala-His-Gly-Lys-Lys-Val-Leu-Gly-Ala-Phe-Ser-Asp-Gly-Leu-Ala-His-Leu-Asp-Asn-Leu-Lys-Gly-Thr-Phe-Ala-Thr-Leu-Ser-Glu-
      100          110          120
Leu-His-Cys-Asn-Lys-Leu-His-Val-Asp-Pro-Glu-Asn-Phe-Arg-Leu-Leu-Gly-Asn-Val-Leu-Val-Cys-Val-Leu-Ala-His-His-Phe-Gly-Lys-
      130          140          146
Glu-Phe-Thr-Pro-Pro-Val-Gln-Ala-Ala-Tyr-Gln-Lys-Val-Val-Ala-Gly-Val-Ala-Asn-Ala-Leu-Ala-His-Lys-Tyr-His-COO
  
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The proteins were aminoethylated (reacts with cysteine and makes it look like lysine) and subjected to trypsin hydrolysis. The resulting peptides were separated by a combination of electrophoresis and paper chromatography and compared to normal hemoglobin. In each case only one peptide differed from the normal hemoglobin peptide map. The amino acid composition of the affected peptides is shown in the table below.

Source of Variant Peptide	Amino Acid Composition								
	Arg	Asp	Glu	Pro	Gly	Ala	Val	Leu	Cys
Hemoglobin G-Couthatta	1	2	1	0	3	2	3	1	0
Hemoglobin Southampton	0	1	0	1	1	0	2	2	1

1. From the above compositions and knowledge of the procedures to obtain the peptides, deduce the location of each peptide in the overall sequence. Describe how you arrived at your answer. How sure are you of your assignment?
2. What is the amino acid substitution that has taken place? If possible, give the position number and the amino acid that normally occurs at that position. Would the substitution affect the electrophoretic mobility of the intact hemoglobin molecule?

Problem written by Deborah Mowshowitz and modified by H. B. White