Q1A. (10 points) Suppose a second mutation occurs in HbS, which changes the amino acid residue Phe85 to Glu. What effect would this have on sickling of red blood cells? Explain your answer with an illustration to support your reasoning. (Hint: Use a screenshot from the interactive images in http://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/hemo/hemoglobin.html and find a suitable view to explain your answer).

Representative Full Answer:
In HbS, the mutant Val6 of a β subunit in one hemoglobin tetramer minimizes contact with solvent H2O by packing into a complementary hydrophobic surface pocket of a β subunit of another hemoglobin tetramer. Mutating the hydrophobic Phe85 to Glu would change the hydrophobicity/polarity and charge of the amino acid side chain in the hydrophobic patch. In the HbS deoxy form, two chains of the mutant Hb beta chains interact so that Val6 (shown in magenta in the left figure and light gray on the right figure) of one chain is closely interacting with Phe85 (shown in white in the left figure and dark grey in the right figure) of another chain.

This substitution of Phe85 with Glu would not favor the above hydrophobic interactions with the mutated Val6 from the neighboring hemoglobin molecule’s Hb beta chain. Thus, this additional mutation may have an anti-sickling effect and prevent fiber formation.

Q1B. (10 points) Suppose a second mutation occurs in HbS, which changes the amino acid residue Ala70 to Val. What effect would this have on sickling of red blood cells? Explain your answer with an illustration to support your reasoning. (Hint: Use a screenshot from the interactive images in http://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/hemo/hemoglobin.html and find a suitable view to explain your answer).

Representative Full Answer:
In HbS, the mutant Val6 of a β subunit in one hemoglobin tetramer minimizes contact with solvent H2O by packing into a complementary hydrophobic surface pocket of a β subunit of another hemoglobin tetramer. Mutating the hydrophobic Ala70 to Val would neither change the hydrophobicity/polarity nor the size of the
amino acid side chain in the hydrophobic patch. In the HbS deoxy form, two chains of the mutant Hb beta chains interact so that Val6 (shown in light gray on the right figure) of one chain is closely interacting with Ala70 (shown in dark grey in the right figure) of another chain.

This substitution of Ala70 with Val would still favor the above hydrophobic interactions with the mutated Val6 from the neighboring hemoglobin molecule’s Hb beta chain. Thus, this mutation will still result in fiber formation and the sickling of the cells.

Grading Rubric:
Biochemical content needs to include:
Group 1: indication for the polarity and charge switch (F85 → E85) of the amino acid due to mutation
Group 2: indication for the preservation of the polarity (nonpolar character) and size of the amino acid due to mutation (A70 → V70)
Both groups: Explicit statement for the cause of sickling fiber formation requires both the hydrophobic Val6 and the corresponding hydrophobic patch on the other chain.

Notes:
1. if the description does not include that the patch and V6 are on different beta chains answer is marked as “a minor mistake/omission”
2. If only one of the two highlighted properties are mentioned answer is marked as “a minor mistake/omission”

Biochemical Support needs to include:

Reasoning:
For Group 1: Accurately worded description of how losing the complementary hydrophobic patch due to the switch of POLARITY AND CHARGE would have an “anti-sickling effect” by no longer having the thermodynamic drive to form associations/fibers.
For Group 2: Accurately worded description of how having a SIMILAR POLARITY AND SIZE for the mutant amino acid would not alter the outcome since there would still be the thermodynamic drive to form associations.

Evidence:
Both Groups:
A figure that clearly shows the packing of two components required for fiber formation (Val 6 and the hydrophobic patch (A70, F85, and L88) or at least F85 (for group A) or A70 (for group B) to exclude H2O. 

Notes: 
1. If there is no annotation or figure legend for the figure and/or cross reference to the text the answer is marked as “a minor mistake/omission”.
2. If no figure is included or the figure does not support the correct idea the answer is marked as “a major mistake/ omission”.
3. For this question you were not expected to correlate this mutation with the O2 binding ability of the mutated hemoglobin chain to get full credit. However if you chose to do it, you needed to do it correctly. This hydrophobic patch makes hydrophobic contacts with the heme group and F85 and L88 are directly connected to H87 (the proximal histidine). Hence this mutation will impact O2 binding. However, sickling is due to the surface contact of the patch residues with the valine which happens on the opposite side of the patch. So if you described a link by stating the change in O2 binding will change the surface exposure characteristics of the patch and hence will impact sickling the answer may be acceptable (if the reasoning is correct) however if you said “because O2 doesn’t bind (or binds more) sickling happens or doesn’t happen” the answer is marked as “a major mistake/ omission”.