INTERMOLECULAR CONTACTS OF DEOXYHEMOBLOGIN S: A HYPOTHESIS AND SEARCH FOR POSSIBLE ANTI-SICKLING AGENTS

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SUMMARY: A single replacement of β6 glutamic acid in each of the β chains of hemoglobin (Hb) A by valine in Hb S should have only a local conformational change near the N-terminals. It is hypothesized that the first turn of helix A (β4-6) is unwound and residues β1-6 of one β chain of a Hb S molecule contacts the nonhelix EF region of residues β77-84 (between helices E and F) of another Hb S molecule. The aggregation proceeds in both directions of the two β chains leading to single filaments, followed by lateral aggregation into 6-stranded fibers and gelation of sickle-cell hemoglobins. We propose that small peptides which mimic portions of the binding sites might interfere with the gelation, thus becoming possible anti-sickling agents.

Sickle cell hemoglobin (Hb) S differs from normal Hb A in two aspects: First, out of 574 amino acid residues in the globin only two β6 valine residues in Hb S replace glutamic acid residues in Hb A; the primary sequence at the N-terminal is:

1				5				10
Val	His	Leu	Thr	Pro	Val	Glu	Lys	Ser	Ala ...

Second, only deoxygenated Hb S in concentrated solution gels, especially at relatively high temperature such as 37°C.

Current models. Murayama (1, 2) first suggested that the two β6 valine side groups of a deoxyHb S molecule protrude into complementary binding sites of the α chains of another molecule, leading to single filaments (six filaments forming a hollow molecular cable). From electron microscopy and X-ray diffraction studies, Finch et al. (3) have proposed that the structure of the fiber is made up of 6 helical filaments and their molecules are in longitudinal register so that they form flat, stacked hexagonal rings, each ring being rotated by 7.3° relative to the one below it. These authors suggest no specific role for the β6 mutation sites. In the model of

Edelstein et al. (4), the fiber consists of a sextuple helix in terms of the long striations and a double helix of short striations with 6.4 molecules per turn. More recent studies seem to indicate some uncertainties in this model (5).

The polarized absorption studies of Hofrichter et al. (6) indicate that the orientation of the pseudo-2-fold x-axis of the deoxyHb S molecule should be restricted to within 22° of the fiber axis. This rules out the Murayama model. These authors favor the model of Finch et al. (3); they suggested three possible structures requiring one or two intra-ring contacts of β6 sites.

Working hypothesis. We propose the following stereochemical mechanism of gelation:

A. Local conformational change near the β6 region. The β6 region lies on the surface of the Hb molecule. Residues β1-3 are nonhelical and residues β4-19 form helix A (7). A single replacement at the two β6 positions should not drastically alter the overall protein structure. But the specificity of the gelation of deoxyHb S does require some minor conformational difference between Hb S and Hb A. We propose that in Hb S the first turn of helix A is unwound and residues β1-6 can adopt a Pauling-Corey β-sheet (Fig. 1) at the N-terminal. These residues can rotate outward to the solvent and make contacts with certain residues in another Hb molecule. An ideal β-sheet with maximum hydrogen bonds is not required, but there must be one or more contacts involving the N-terminal residues.

Proline is a helix-breaker because its imide nitrogen cannot form a hydrogen bond (for that matter, it is a β-sheet-breaker too). But β5 Pro is tolerated at the beginning (or end) of a helix which does not have hydrogen bonds. Thus, proline is a helix- and β-"indifferent" residue. We hypothesize that valine is a weaker helix-former than glutamic acid and Pro-Val makes it possible to unwind the first turn of helix A.

Direct evidence must ultimately come from X-ray diffraction studies.

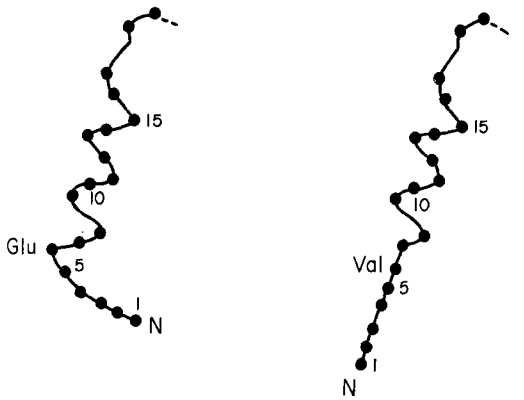

Figure 1. The proposed conformation of the N-terminal residues of the β chains in hemoglobin S. The first turn of helix A is unwound and residues β1 to β6 become unordered and can adopt a single β-sheet. These residues can rotate rather than be fixed as shown. The corresponding conformation for hemoglobin A is included for comparison.

The structure of deoxyHb S at 5 Å is being solved (8). Even this information might not be enough to describe the aggregated deoxyHb S. The stability of a helix and a β-sheet is marginal as compared with the unordered form. It is still conceivable that either Hb S and Hb A have the same N-terminal conformation or residues β1-6 of Hb S are unordered prior to aggregation.

The Hb A molecule contains 79% helix and no β-sheet. Our hypothesis predicts a maximum of about 2% β-sheet for the Hb S molecule with a 1% reduction in helical content. Experimentally, it would be difficult, if not impossible, to detect such small changes in conformation by current physical methods such as circular dichroism. Our hypothesis, however, is not inconsistent with the conformational predictions based on amino acid sequences. For instance, Lewis et al. (9) have computed the helix probability profiles in proteins and assigned glutamic acid as a helix-former and valine as a helix-"indifferent" residue. More recently, Chou and Fasman (10) have enumerated the conformational parameters and listed glutamic acid as the strongest helix-former and β-sheet-breaker. But valine is according to their published data the second strongest β-sheet-former and only a moderate

helix-former. Thus, residues β1-6 of Hb S might have a higher probability of forming a β-sheet than a helix (β7 Glu and β8 Lys are both β-sheet-breakers). These methods of predictions involve many assumptions (to be discussed elsewhere). Any agreement between our proposed conformational change and that based on empirical predictions could be fortuitous.

B. Possible contacts between β1-6 and β77-84. Deoxygenation of Hb A causes a change in its quaternary structure (11). It is roughly true that the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ halves in the Hb molecule slide past each other (actually the largest shift is in the $\alpha_1\beta_2$ (or $\alpha_2\beta_1$) contacts). Upon deoxygenation the distance between the two hemes of the β chains is increased by about 7 Å, while that of the α chains come closer by about 1 Å (the distance between the hemes in α_1 and β_1 (or α_2 and β_2) chains changes very little). As a result, the regions of helices E and F and nonhelix EF of the two β chains shift away from each other in the deoxy-state, noting that the helix A is juxtapositioned to helix E. Since deoxygenation triggers the formation of Hb S fibers, it is tempting to suggest that the nonhelix EF (β77-84) region might be a candidate for contact with β1-6 of another molecule:

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          77          80              84
... His Leu Asp Asn Leu Lys Gly Thr ...
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The geometric arrangement is such that the deoxy-form makes such a contact possible.

DeoxyHb C (Harlem) with two replacements (β6 Glu→Val and β73 Asp→Asn) also gels, but its minimum gelling concentration is much higher than that of deoxyHb S and mixtures of deoxyHb S and deoxyHb Korle Bu (β73 Asp→Asn) gel at concentrations much higher than those of deoxyHb S and deoxyHb A. Thus, Nagel and Bookchin (12) have proposed that the β73 region of one of the β chains is involved in an intermolecular contact. Note that residue β73 is located at the last turn of helix E. The movement of the nonhelix EF region is restricted by the geometry of helices E and F. It is not necessary to assume an unwinding of residues β73-76, although this possibility cannot be ruled out.

C. Polymerization of deoxyHb S. The process can of course proceed in both directions leading to single filaments (see also Ref. 13). The β1-6 region of one of the β chains contacts the β77-84 region of another molecule and the β77-84 region of the other β chain contacts the β1-6 region of a third molecule. The single filaments in turn form the 6-stranded fibers (here we do not speculate on any inter-filament or intra-ring contacts). Finch et al. (3) found that deoxyHb A also polymerized into filaments, "but with certain important differences. ... They did aggregate side by side in longitudinal register, but the number of filaments aggregating was irregular." Thus the gelation of Hb S dictates a specific stereochemical mechanism of intermolecular contacts.

Since the β1-6 region constitutes only about 2% of the Hb molecule, it is not too surprising that a high Hb concentration is required for nucleation, followed by rapid gelation. We are aware of the fact that replacements or modifications of residues in other regions of the β chains or several regions of the α chains could enhance or diminish gelation. Molecular contacts in these regions must also be involved in gelation but it is premature to pinpoint their detailed structures at present.

The gelation of mixtures of deoxyHb S and non-S deoxyhemoglobins (Hb A, Hb C (Harlem) and Hb Korle Bu) can be attributed to the formation of hybrid Hb tetramers (12, 14, 15). Some of the tetramers $\alpha_2^A\beta_2^A$ could also be tolerated in a gel, even though they may only have one key molecular contact in the β77-84 region. Recent reports (16-18) that liganded form such as CN-metHb A can also participate in gelation by forming hybrid tetramers with Hb S have raised the question as to the necessity of the Hbs in the deoxy-quaternary structure for gelation to occur. We must await more detailed studies before any conclusion can be drawn.

Possible anti-sickling agents. Any hypothesis is by necessity an oversimplification, but it is useful when it suggests the design of experiments to test its validity. In particular, we propose the use of oligo-

peptides, which mimic portions of the amino acid sequence of Hb S such as residues β1-6 (work in progress), as possible anti-sickling agents. Such oligopeptides or even a single amino acid or its derivative may compete for the binding sites between deoxyHb S molecules and thereby interfere with the gelation of Hb S. The function of an anti-sickling agent is essentially to raise the minimum gelling concentration so high that deoxyHb S would not gel under physiological conditions. Much of current interest has been concentrated on chemical modifications such as carbamylation (19), which will equally modify normal Hb A. In this work we present a different approach, that is, the search of "inhibitors" without chemical modifications. We also realize that the transport of any compound across the red blood cells will be far more problematic than the studies of the effect of such species on Hb S alone. Nevertheless, the understanding of the stereochemical mechanism of gelation of deoxyHb S is fundamental to our search for a possible therapy and represents a first step to combat this hereditary blood disorder.

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