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Structure of Fibers of Sickle Cell Hemoglobin

(blood/anemia/electron microscopy/double helix)

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ABSTRACT **Electron microscope studies have been conducted on individual fibers of human deoxyhemoglobin S (sickle cell hemoglobin). The fibers are obtained by injection of gelled samples into a large excess of glutaraldehyde, which quickly stabilizes the fibers by crosslinking. The fibers are negatively stained with phosphotungstic acid or shadowed with platinum–carbon. The fibers are approximately 200 Å in diameter, and display long and short helical striations with an opposite handedness. The long striations occur at an angle of about 15° from the fiber axis, and complete one turn around the helix at a distance of about 2×10^3 Å along the fiber axis. The short striations occur at an angle of about 80° from the fiber axis, with a spacing of about 65 Å, and complete one turn around the helix at a distance along the fiber axis of about 130 Å. The structure of the fiber appears to be a sextuple helix in terms of the long striations, and a double helix in terms of the short striations. The shadowed samples are consistent with a left-handed screw sense for the short striations, thus implying a right-handed sense for the long striations. A structural model incorporating these features is compatible with the atomic structure of hemoglobin, with individual molecules oriented with their dyad axis of symmetry perpendicular to the fiber axis and their α_1–β_1 pseudo-dyad axis roughly parallel to the fiber axis. This orientation places the two β-6 regions of each molecule (sites of the sickle cell mutation) in contact with the β-6 regions of the molecules above and below along the long striations. Both the long and short striations are accounted for by individual hemoglobin molecules arranged in double helical arrays with 6.4 molecules per turn in each array.**

The existence of sickle-shaped erythrocytes in human blood has been known since the early part of this century, and in 1927 Hahn and Gillespie (1) correlated sickling with low oxygen tension. The propensity for sickling was identified with an alteration in the hemoglobin molecule by Pauling, Itano, Singer, and Wells (2) and in 1957 Ingram found that sickle cell hemoglobin differed from normal hemoglobin by a change of one amino acid residue in the beta chains (3). A glutamyl residue in the β-6 position was replaced by a valyl residue (3). This altered hemoglobin is now generally known as hemoglobin S. The sickling phenomenon is caused by an aggregation of hemoglobin S molecules into long fibers, which orient to deform the erythrocytes and impair circulation. Although some general structural information is available, the precise structure of the fibers remains unknown. Earlier electron microscope studies have revealed fibers roughly 200 Å in diameter (4–7) and x-ray diffraction measurements have recently been reported (8) that indicate a helical arrangement of the molecules in the fibers, with a repeat distance of 64 Å. However, a detailed solution of the x-ray diffraction patterns has not yet been achieved. Any final structural model should also take into account the results of optical dichroism (9, 10) and magnetic orientation (11) measurements, as well as the geometry of the individual hemoglobin molecules (12). In order to provide additional structural information on the fibers of hemoglobin S, studies on the structure and assembly of fibers have been begun in this laboratory by analytical ultracentrifugation and electron microscopy. Our initial results from electron microscopy have revealed several striking structural features that permit some conclusions to be drawn on the arrangement of molecules in the fibers of hemoglobin S; these results are presented here.

METHODS

Hemoglobin S was obtained from the blood of individuals homozygous for the sickle cell mutation. Blood cells were washed three times in 0.9% saline and lysed with an equal volume of distilled water. Membranes were pelleted by centrifugation, and the remaining solution was concentrated to 18% (w/v) by pressure dialysis in an Amicon ultrafiltration apparatus. Solutions were deoxygenated by alternately evacuating and equilibrating with nitrogen. Gelled solutions were forced through a syringe needle into nitrogen-saturated solutions of 8% glutaraldehyde (Biodynamics Research Corp., Rockville, Md.) in distilled water. The mixture was then centrifuged to concentrate heavy material and the pellet was resuspended in a small volume of water and agitated with a sonicator to obtain dispersal. The final solution was picked up on a carbon-coated grid and stained with 2% phosphotungstic acid. The grids were examined with an AEI electron microscope (EM6B) and the photographs were recorded at a calibrated magnification of 64,130. Shadowing was performed with a Materials Research Corp. apparatus (series B-4) with platinum–carbon (Ladd Research Industries, Inc., Burlington, Vt.).

RESULTS

Examination of the electron micrographs of several hemoglobin S samples crosslinked with glutaraldehyde and negatively stained with phosphotungstic acid consistently revealed individual fibers with marked helical striations (Fig. 1). Samples displayed the crosshatched marks expected for a helical structure with the front and back superimposed (13). In order to extract structural data from these micrographs, image reconstruction and optical filtering studies (14) have been initiated; they will be described elsewhere. However, the samples reveal considerable structural information without image reconstruction. Measurements of the features from several micrographs can be summarized as follows. Fibers are approximately 200 Å in diameter, with short helical turns

observed at a spacing of 60–70 Å (average about 65 Å). The 200-Å diameter should be considered an upper limit since apparent swelling due to the staining process, possible cross-linking of individual hemoglobin molecules to the fibers by glutaraldehyde, and flattening of the fibers on the grids would all tend to elevate this value. The long striations each complete one turn (360°) about the fiber axis at a spacing of about 2×10^3 Å. The short striations are inclined to give one complete turn for each striation at a spacing of about 130 Å. Individual globules (presumably hemoglobin tetramers) are present in adjacent positions of a single turn at a surface spacing of about 100 Å. The number of molecules per turn is thus given by (diameter/spacing) $\times \pi$ and, in this case, is about 2π or about 6.3. Examination of helical regions where both the long and short striations are present indicate that the two types of striations occur at roughly right angles. The long striation lies about 15° from the fiber axis, and the short striation lies at an angle of about 80° from the fiber axis (see Fig. 1). Where globules can be observed that lie on both the long and short striations, the features suggest that the long and short striations possess opposite handedness. From the electron micrographs obtained with negatively stained samples, it is not possible to establish the absolute handedness without tilting of the electron microscope stage, since both left- and right-handed examples of each type of striation can be observed (Fig. 1) depending on whether the striations seen arise from the front surface or the back surface of the fiber. However, with samples prepared by shadowing, only the front surface is highlighted and, as shown in Fig. 2, results with shadowed fibers show a striation at an angle of about 80° from the fiber axis consistently rising to the left. Therefore, the short striation appears to be left-handed, implying that the long striation is right-handed.

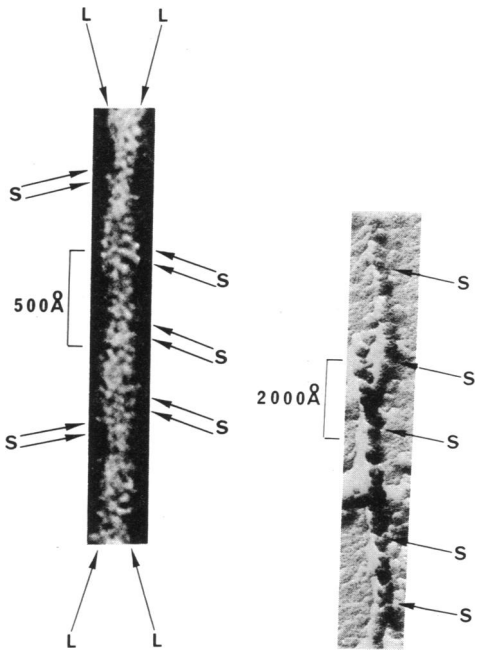

FIG. 1 (*left*). Electron micrograph of hemoglobin S cross-linked with glutaraldehyde and negatively stained with phosphotungstic acid. Long and short striations are apparent. The long striations (indicated by *L*) occur at an angle of 15° from the fiber axis. The short striations (indicated by *S*) occur at an angle of 80° from the fiber axis.

FIG. 2 (*right*). Electron micrograph of hemoglobin S cross-linked with glutaraldehyde and shadowed with platinum–carbon. The fibers are greatly broadened compared to the negatively stained material (Fig. 1), due to a piling-up of the shadowing agent. However, striations are apparent at the same angle from the fiber axis (80°) as the short striations in the negatively stained material. In the shadowed sample, the short striations (*S*) have a left-handed screw sense.

DISCUSSION

The electron micrographs of individual fibers of sickle cell hemoglobin give clear indications of a helical structure. Possible helical structures for hemoglobin S have been suggested before (4, 15–17), although no direct experimental evidence was then available. Magdoff–Fairchild and coworkers (8) found x-ray diffraction data consistent with a helix, although they were unable to provide a detailed structural interpretation. The work presented here is still at an early stage and is insufficient to indicate a unique structure for the fibers with complete assurance. Nevertheless, adequate features are evident for a tentative structure to be deduced. Refinement of the electron micrographs and possible correlations with the x-ray diffacton data should eventually permit an evaluation of this structure and more precise specification of the orientation of the individual hemoglobin molecules.

The model proposed is based on the regular features of the helical striations of the fibers. The long striations, which occur at an angle of about 15° to the fiber axis, complete one turn at about 2×10^3 Å. Since a spacing of about 65 Å is observed between short striations, and this distance corresponds to one hemoglobin molecule (12), each long striation has about 2×10^3 Å/65 Å or about 30 molecules per turn. Thus, adjacent molecules along the long striation are staggered by about 0.03 turn, or by about 12°. The short striations occur at an angle (about 80° from the fiber axis) that is too steep to be compatible with a single (one-start) helix and suggests a double (two-start) helix. Each short striation completes one turn around the fiber at a spacing of 130 Å along the fiber axis and each hemoglobin molecule is staggered from the one below or above it on the same short striation by about $2 \times 12°$ or about 24°, corresponding to 6.4 molecules per turn for each short striation, in good agreement with the value (about 6.3) deduced from the fiber dimensions (see above).

The structural parameters have been incorporated into a double helix with 6.4 molecules per turn. The molecules are also arranged to account for the long striations in terms of an apparent sextuple helix. Two views, top and side, are shown in Fig. 3. The hemoglobin molecules are aligned with their dyad axes perpendicular to the fiber axis and the α_1–β_1 pseudo-dyad axes parallel to the fiber axis as suggested by Perutz (18). In addition, the molecules are oriented with their beta chains facing the center of the helix, in order to bring the β-6 valine into a position for interactions with other molecules. As seen in the top view, each molecule is staggered from the one below it by 12°, from the adjacent molecule by 56°, and from the one below along the same striation by 24°. Thus, the two striations interpenetrate in typical double-helical fashion. As seen in the side view (surface lattice), each molecule is offset slightly from those above and below to account for the long striation (15° from the fiber axis). Molecules along the short striation (80° from the fiber axis) are elevated by (130 Å per turn)/(6.4 molecules per turn) or about

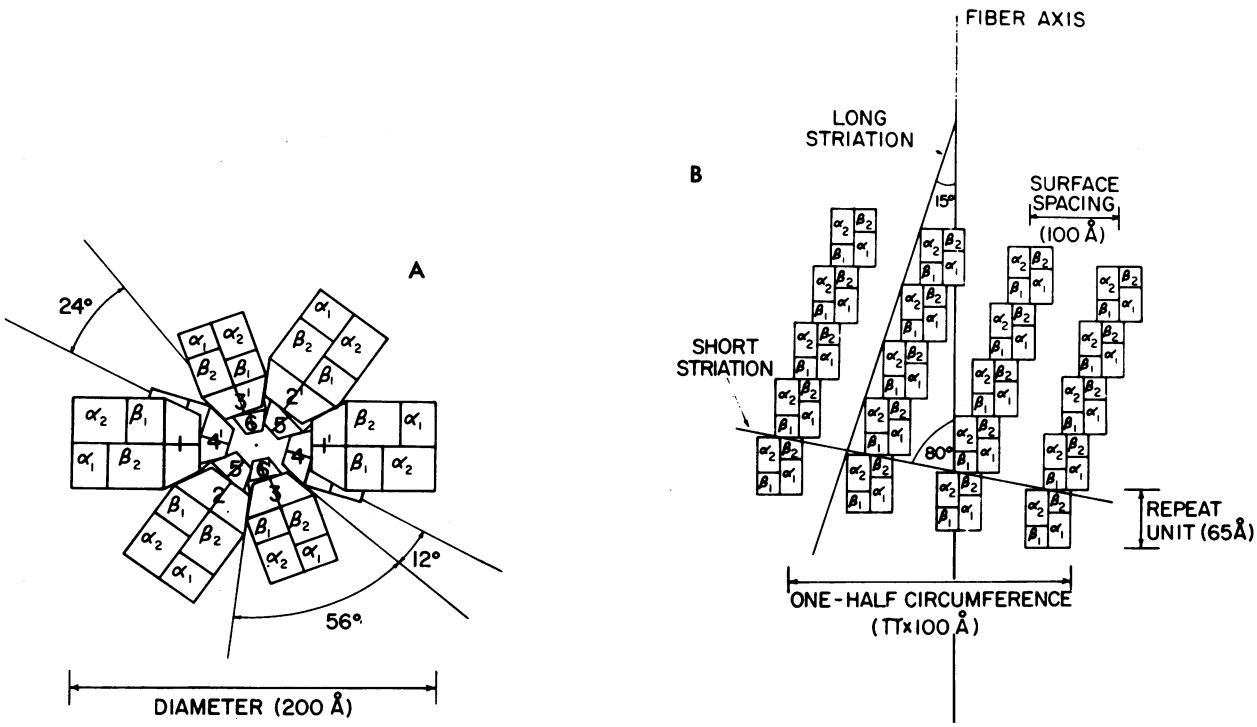

FIG. 3. Model of hemoglobin S fibers. *A*. Top view (looking down the fiber axis). *B*. Side view (surface lattice). Molecules are oriented with the α_1–β_1 pseudo-dyad axis parallel to the fiber axis and the β chains facing the center of the fiber. Therefore, the α_1 and β_1 subunits are reduced in size in the top view (*A*) to reflect the fact that they lie somewhat below the α_2 and β_2 subunits in this perspective. Similarly, both β subunits are reduced in size in the side view (*B*) to reflect the fact that they lie somewhat behind the α subunits in this perspective. The short striations (indicated in the side view of the surface lattice) form a double helix, as shown in the top view. The intertwining of two arrays (with 6 molecules in each array) is shown in (*A*) with the molecules of one array in descending order indicated by the numbers *1–6* and the molecules of the other array indicated by the numbers *1′–6′*.

20 Å per molecule. Since the side view is a surface lattice (representing the surface of the fiber opened and flattened), the contacts along the short striations (seen in the top view) are not in evidence since they occur near the interior of the helix, not at the surface. However, the contacts of the long striation are retained in this perspective.

In the side view shown (Fig. 3) the α_1–β_1 pseudo-axis is fixed exactly parallel to the fiber axis, although some rotation about the true dyad axis (perpendicular to the fiber axis) is still compatible with a 65-Å spacing and other structural requirements of the fibers (18). The uncertainty in the orientation of the α_1–β_1 pseudo-dyad axis leads to an uncertainty in the contact sites between hemoglobin molecules. With the α_1–β_1 pseudo-dyad axis parallel to the fiber axis, as shown in Fig. 3, contacts between molecules along the long striations involve regions of the beta chains in the vicinity of the β-6 position. The β-6 residue is also in the general location for a possible contact with the β-73 region of the hemoglobin molecules above and below. Substitutions at this position alter gelling properties, as in hemoglobin C Harlem (19). However, the exact region of contact is highly dependent on the α_1–β_1 pseudo-dyad axis, and will be altered markedly if the axis is rotated slightly from the position parallel to the fiber axis. The correct orientation of the α_1–β_1 axis may be difficult to resolve, particularly in a way that explains the data arising from gelling experiments conducted with mixtures of hemoglobin S and other mutant human hemoglobins (17). One approach to this problem currently being pursued in our laboratory is to determine the sites on the individual hemoglobin molecules that are crosslinked. Information of this type may permit the orientation of the molecules to be fixed within more specific limits. Similar studies in progress involving crosslinking of solutions soon after gelling is initiated (by elevation of the temperature) may also reveal the principal interactions in the fiber assembly process and provide some clues as to whether the long striations or the short striations represent the dominant interactions. Through such studies, more detailed model building, and refinements of electron microscope and x-ray diffraction results, the remaining uncertainties in the structure of the fibers should be eliminated. The availability of a precise structure would facilitate the design of antisickling agents along the lines already initiated (20, 21), and might contribute to the treatment of sickle cell disease.

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