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DOUBLE FILAMENTS IN FIBERS AND CRYSTALS OF DEOXYGENATED HEMOGLOBIN S

Beatrice Magdoff-Fairchild and Celia C. Chiu, *Hematology Division, St. Luke's Hospital Center, and Department of Medicine, College of Physicians and Surgeons, Columbia University, New York. 10025 U.S.A.*

INTRODUCTION

Sickle cell hemoglobin (HbS) molecules in solution or in SS erythrocytes (those from individuals homozygous for the sickle hemoglobin gene), when deoxygenated, aggregate to form fibers that pack into paracrystalline arrays. The diminished oxygen affinity of HbS is produced by the polymerization, and the distortion of the pliant erythrocyte membrane in sickle cell disease results from the elongation of polymers and their subsequent alignment. One of the important problems to be solved in sickle cell disease is the definition of the intermolecular interactions that stabilize the fiber structure. Knowledge of these interactions might lead to the design of stereospecific antisickling agents for clinical use that could inhibit polymerization or could at least destabilize the fiber.

RESULTS AND DISCUSSION

In general, molecular structure and fiber architecture must first be known to find the interactions of one molecule with its neighbors in fiber or in other helical structures. In this particular case, since the structure of deoxy-hemoglobin S is known to 3 Å resolution (1), and since the fiber diffraction pattern shows remarkable similarity to the patterns of monoclinic crystals of deoxy-HbS (form I) (2), the interactions can be determined without complete knowledge of molecular packing in the fiber. By comparing fiber and crystal diffraction patterns, we have been able to establish that the basic structural unit of the fiber consists of double filaments. As in the crystal, one filament is related to its neighbor by the operation of a twofold screw axis located between the filaments and parallel to the fiber axis. Establishment of the partial structure of the fiber leads to the identification of interactions along and between the filaments similar to those in the crystal. The major stabilizing interaction is between the substituted amino acid residue in HbS, Val$1\beta 6$, on one molecule and the hydrophobic residues Phe$\beta 85$ and Leu$\beta 88$ on the neighboring molecule in the adjacent filament (3). Within each filament, hydrogen bonds and van der Waals forces account for most of the interactions.

Another monoclinic crystal (form II) has been identified in capillaries of tightly packed

TABLE I
CELL DIMENSIONS (IN Å) OF CRYSTALLINE FORMS OF DEOXY-HbS, AND FIBER PARAMETERS

	Monoclinic (1,2,3,4)	Triclinic	Fiber (2)
a	63.3	63.8	64
b	185.7	92.5	(Layer line repeat)
c	53.0	53.0	220,110,73,53
α	90.0°	81.0°	(Equatorial reflections)
β	92.7°	92.9°	
γ	90.0°	99.4°	

deoxygenated SS erythrocytes that initially contained aligned fibers (4). Crystals of forms I and II have the same space group, $P2_1$, and cell dimensions (Table I). The intensity distributions, however, of the hk0 reflections of these two crystals differ markedly, although the 0k0 reflections are the same. These similarities in space group and cell dimensions suggest that the molecular packing in both crystals is essentially the same, and the differences in the hk0 zones indicate that the difference in structure of these forms resides only in the x-coordinate of the molecules.

The combined rotation diagrams of forms I and II resemble the fiber pattern more closely than would either one alone. The fiber pattern has a strong 2nd order meridional or near-meridional reflection, while the 200 reflection is weak or absent for form I and strong for form II. The strong 310 reflection of form I can account for the strong apparent 3rd order meridional maximum on the fiber pattern when nonparallelism of fiber alignment is taken into consideration. In form I, the 310 reflection is strong as in the fiber, while in form II this reflection is weak. Since the crystal structures contain a pair of antiparallel double filaments, the fiber must also consist of antiparallel double filaments. One quadruple set of filaments would be similar to that found in form I, with the same displacement of the double filaments with respect to their neighboring antiparallel filaments, while the second quadruple set would have a different relative displacement of the antiparallel pair of double filaments than in form I.

Supportive evidence that antiparallel pairs of double filaments comprise the fiber comes from a third crystalline form. This crystal (form III) is triclinic and has been found in only two cases of the great many diffraction patterns of preparations of deoxygenated SS erythrocytes that have been recorded. Diffraction patterns of form III have also been observed when crystals are prepared from deoxygenated HbS solutions as described elsewhere (5). This crystalline form contains only two molecules in the unit cell. Comparison with the cell dimensions of the monoclinic forms (Table I) suggests that the two molecules in this crystal pack to form double strands similar to those found in the monoclinic crystals. The rotation diagram of this form, however, although it resembles that of the fiber pattern, does not show the striking similarities to the fiber pattern that diagrams of forms I and II indicate. From these comparisons as well it appears that antiparallel pairs of double filaments, rather than only one set of double filaments, is the basic structural unit of the fiber.

The question arises how a fiber consisting of antiparallel pairs of double filaments that form a helix of large repeat and a closed cylindrical surface can resemble so closely the infinite 3 dimensional arrays as they exist in crystalline structures. The answer to this was sought with techniques of electron microscopy. Preparations of fibers in SS erythrocytes were examined where fiber to crystal transition occurred.

No stepwise sequence in the transition was found despite the close structural similarities of fiber and crystals. Rather, simultaneously with the appearance of crystallites, a spectrum of structures that included microtubules, and large diameter solid cylinders were observed. A loss or addition of only one double filament in the physiological fiber structure within the erythrocyte could account for this polymorphism. These results suggest that the HbS polymers disaggregate and that monomers and partial polymeric structures reassemble to form a variety of structures. Eventually, ordered crystals grow at the expense of monomers and other polymers and equilbrium is reestablished.

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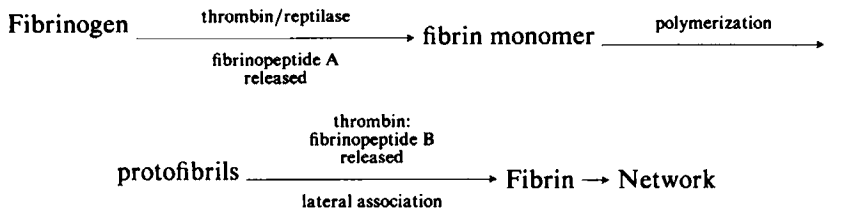
FIBRIN FORMATION AS A BIOLOGICAL ASSEMBLY PROCESS

Roy Hantgan and Jan Hermans, *Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514*
Walter Fowler and Harold Erickson, *Department of Pharmacology and Anatomy, Duke University, Durham, North Carolina 27706 U.S.A.*

Fibrin assembly in vivo is initiated by the limited proteolytic action of the enzyme thrombin on the 340,000-dalton soluble protein fibrinogen. In this communication, attention will be focused on the molecular mechanism of fibrin formation.

The results of a light scattering study of fibrin assembly (1) support the following (schematic) mechanism:



By employing non-rate limiting concentrations of the activating enzyme, it has been possible to separate (in time) the proteolytic steps which initiate gelation from the two subsequent