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

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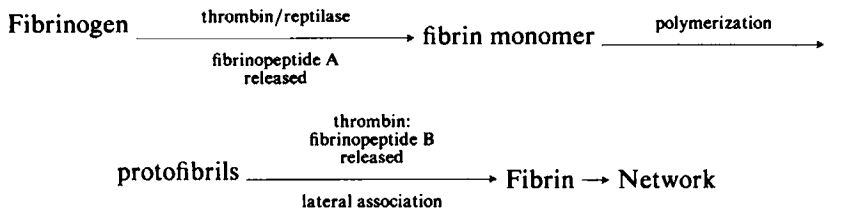
Fibrin assembly in vivo is initiated by the limited proteolytic action of the enzyme thrombin on the 340,000-dalton soluble protein fibrinogen. In this communication, attention will be focused on the molecular mechanism of fibrin formation.

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

physical steps: polymerization and lateral association, both of which can be directly observed by light scattering. Network formation has been studied by sensitive time-dependent measurement of clot rigidity.

More recently, a new negative staining technique developed to study the molecular structure of fibrinogen in the electron microscope (2) has been applied to follow the time-dependent structural changes characteristic of fibrin assembly. Results of this electron microscope investigation are consistent with the assembly mechanism proposed on the basis of the light scattering kinetic data.

Step 1: Polymerization

Data obtained by stopped-flow light scattering experiments (1) indicate that removal of fibrinopeptide A, by reptilase or thrombin, triggers the biomolecular polymerization of bifunctional monomers to form protofibrils, long thin polymers formed by a half-staggered overlap of fibrin monomers. The kinetics of fibrin assembly have been studied under conditions favoring the formation of thin (0.5 M NaCl, pH 7.4) as well as thick (0.1 M NaCl, pH 7.4) fibers; in both cases two distinct processes (polymerization of monomers to protofibrils and lateral association of protofibrils to form fibers) have been observed by light scattering techniques.

The rate of protofibril formation varies as the inverse first power of the concentration. The data can be fitted to theoretical scattering-vs.-time profiles with a single biomolecular rate constant of 8.2×10^5 M^{-1} s^{-1} for association of two monomers, a monomer and oligomer, and two oligomers. There is no evidence for a nucleation step in the assembly of protofibrils.

In negatively stained specimens of thrombin-treated fibrinogen prepared under high salt conditions and at times before the gel point, long thin fibers are indeed the predominant species seen in the electron microscope. These protofibrils are from two to four molecules thick.

At low salt and fibrinogen concentrations, the protofibril formation period appears in light scattering experiments as a lag time of ~60s, preceding the large scattering change accompanying fiber formation. Electron micrographs of samples obtained during this lag time reveal protofibrils and some thicker fibers with a broad distribution of lengths: at 21 s after the addition of thrombin, 98% of the fibers have lengths <4,000 Å, while by 60 s most are >10,000 Å.

Data obtained from both light scattering and electron microscopy are thus consistent with the proposal that the first step in fibrin assembly is protofibril formation.

Step 2: Lateral Association

The relationship between protofibril and fiber formation has been investigated by light scattering ionic strength jump experiments. In this technique, fibrinogen was activated with thrombin at high salt; at a time when only protofibrils, but not fibers, had formed, the salt concentration was abruptly lowered to thick fiber conditions. The resultant scattering versus time profile showed an immediate rise in intensity without an intervening lag period. The half time for this process varied as the inverse first power of the concentration, indicating that fibers can form from protofibrils without any nucleation step.

Electron micrographs of negatively stained samples obtained under low salt conditions at times beyond the lag time show a gradual thickening of fibers occurring with time. This observation is consistent with the assignment of the second step in fiber formation to the lateral association of protofibrils into thicker fibers.

Step 3: Formation of a Network

Gelation corresponds to the formation of a network of fibers extending throughout the solution. The results of light scattering and clot rigidity measurements support the proposal that gelation of fibrin is a necessary outcome of the (irreversible) assembly of fibers from very long protofibrils. That is, interfiber connections (network branchpoints) will inevitably form in a solution of growing protofibrils or fibers that are forming lateral associations at multiple random points along their length. Fibrin structure at the branchpoints is then proposed to be the same as elsewhere, except that on one side of the branchpoint a portion of the fiber bends away from the remainder (1).

Electron microscope studies of samples obtained at times beyond the light scattering lag time revealed fibers with the cross-striations characteristic of mature fibrin (3). Interfiber connections were found even at early times of assembly.

At times when the light scattering changes are nearly complete, not only are dense fibers observed in the electron microscope, but also long structures that appear to consist of thin fibers loosely arranged in sheets. These loose sheets or fibers also showed the cross-striations characteristic of the dense fibers. Both loose and dense fibers were also observed in samples which included high concentrations of factor XIIIa, which introduces covalent crosslinks into fibrin (rendering the network insoluble even under denaturing conditions). The transformation of loosely to densely packed fibers could contribute to the slow increase in clot rigidity which follows gelation. Alternatively, the continued association of fully formed fibers, which increases the number of interfiber corrections, may be the most important mechanism responsible for the increase in rigidity of the gel.

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A PRESSURE RELAXATION STUDY OF TUBULIN OLIGOMER FORMATION

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When microtubules are cooled to 4°C in vitro, ringlike oligomers are formed. These oligomers consist of ~26 tubulin dimers and of the so called microtubule-associated-proteins (MAPs) (1, 2). The role of these rings is often questioned as they are never observed in vivo.

The assembly of microtubules has been studied in terms of a nucleation and a propagation process. The propagation step has been analysed quantitatively (3–5). About the process of nucleation, however, not very much is known. Quantitative information can be deduced from the apparent stoichiometry coefficient of nucleation. This can be obtained from the linear rate, for which we deduced an equation as follows: Integration of equation 1, leads to a rate