THE RATES OF POLYMERIZATION AND DEPOLYMERIZATION OF SICKLE CELL HEMOGLOBIN

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SUMMARY

The polymerization and depolymerization of concentrated solutions of sickle cell deoxyhemoglobin were initiated by raising and lowering the temperature, and the time courses of the reactions monitored by the change in apparent turbidity. The polymerization reaction exhibits a marked lag phase followed by a rapid increase in turbidity, and is dependent on a very high power of the hemoglobin concentration, roughly the fifteenth. The depolymerization reaction exhibits no such lag, and is much less dependent on concentration. The implications of these results for polymerization models are discussed.

On deoxygenation of erythrocytes from patients with sickle cell anemia, polymerization of the HbS* occurs, which distorts the erythrocytes from their normal biconcave disc form into the characteristic sickle shape, causes a marked loss in deformability and increases the blood viscosity (1). Reoxygenation reverses these effects. Similarly, deoxygenation of purified HbS causes the hemoglobin to aggregate into a nematic gel, which liquefies on reoxygenation (2). The rate at which these polymerization processes occur is of both physiological and physicochemical interest. Extensive sickling will only occur if the residence time of the erythrocytes in the capillary bed is long in comparison with the half-time for the polymerization reaction under physiological conditions. Conversely, sickled erythrocytes will only unsickle if the arterial transit time is long in comparison with the half-time for depolymerization. In short, the extent of intra-vascular sickling, and hence the the likelihood of vaso-occlusion and onset of a sickle cell crisis, may be

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* Abbreviations used: HbS, sickle cell hemoglobin: deoxyHb, deoxyhemoglobin: oxyHb, oxyhemoglobin: IHP, inositol hexaphosphate.

strongly dependent on kinetic factors (3). Studies of the rates of sickling and unsickling have been conducted by a number of investigators (3-6). Further, investigation of the kinetics of polymerization of purified HbS may enable the pathway of aggregation to be determined. It may be possible to decide, for example, whether monomolecular filaments (7,8), discs (9) or some other structures are intermediates in the aggregation process (Fig. 1). We report here preliminary studies aimed at this question.

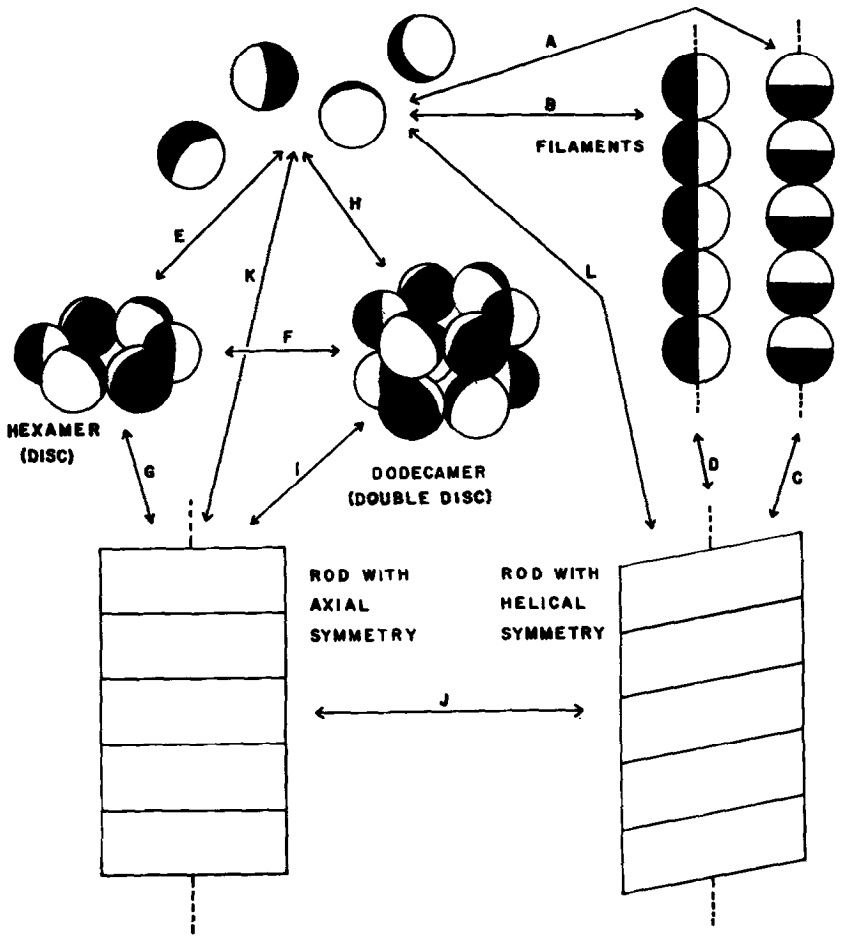

Figure 1. General scheme for the aggregation of deoxyHbS. Individual molecules are shown as spheres in which the shading denotes the β -chains. The models previously proposed proceed via linear condensation into filaments, either reactions A and C (8), or B and D (7). Alternatively, discs (reactions E and G) or double discs (reactions H and I) may be intermediates, as is suggested by the electron microscope observations (9). For illustrative purposes only, the discs and double discs shown have 32 symmetry. Rods with axial symmetry may be converted to rods with helical symmetry, reaction J (9,17).

MATERIALS AND METHODS

Hemolgobin was purified from patients homozygous for HbS, concentrated and deoxygenated as previously described (10). 2 ml fractions of deoxyHbS were placed in a tonometer fitted with a 2 mm path length cuvette, which was inserted in a temperature-controlled water bath with transparent windows through which the monitoring light beam passed. A mean wavelength of 700 nm was selected by a Corning filter. The transmitted light was monitored by a photomultiplier whose output could be directed either to a chart recorder or to a computer-linked data acquisition system (11). Thermal equilibration of the sample could be acheived within one minute of changing the temperature of the water bath.

RESULTS AND DISCUSSION

Gelation of concentrated solutions of purified HbS may be induced either by deoxygenation of oxyHbS, or by raising the temperature of deoxyHbS (8). Although the former approach more closely resembles the physiological reaction, the oxygen must be removed very rapidly which necessitates chemical deoxygenation, mixing concentrated oxyHbS with dithionite. The conventional stopped-flow apparatus is unsuited to this task. Hence, we initiated gelation by the latter approach, rapidly raising the temperature of concentrated deoxyHb solutions from 2^{o} (liquid) to 30^{o} (ultimately gelled). Polymerization was monitored by the increase in apparent turbidity of the sample due to scattering, as a function of time. Typical results obtained at several different initial deoxyHbS concentrations are shown in Fig. 2a. Similarly, de-polymerization was initiated by temperature jumps from 30^{o} (gelled) to 2^{o} (ultimately liquid), with the results shown in Fig. 2b. Identical experiments (results not shown) were carried out on samples free of IHP, with qualitatively similar results.

The most striking feature of the polymerization reaction (Fig. 2a) is the pronounced lag time during which the apparent turbidity is almost constant, followed by a rapid turbidity increase in a pseudo-first order reaction. A

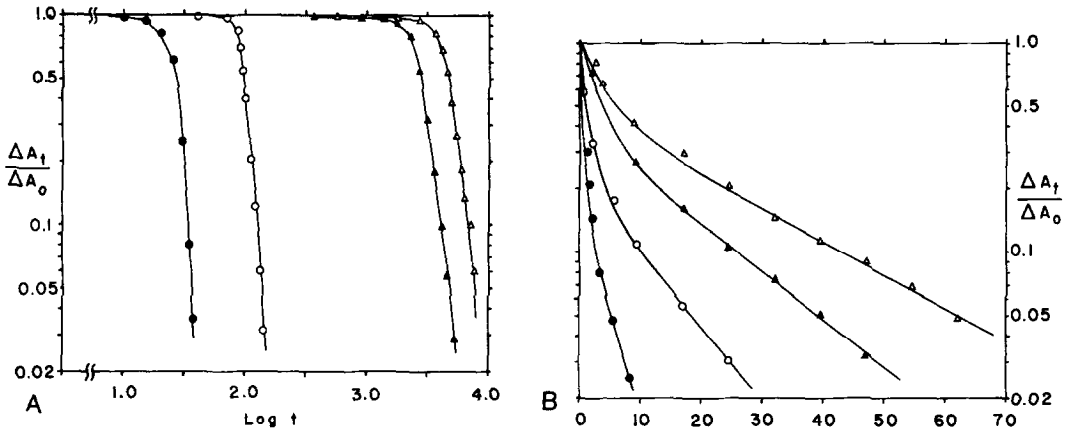

Figure 2. The time course of polymerization and depolymerization of purified deoxyHbS solutions. All runs shown were conducted in 0.1M phosphate, pH 7.0, in the presence of saturating amounts of IHP.

a) Polymerization experiments; temperature jump from 2° to 30° ± 0.2° at time zero. The normalized change in apparent turbidity (see text) is plotted against the logarithm of the time, in seconds, for four initial hemoglobin concentrations: ●, 14.3 mM; ○, 12.5 mM; ▲, 10.5 mM; △, 9.5 mM.

b) Depolymerization experiments; temperature jump from 30° to 2.0 ± 0.2° at time zero. The normalized change in apparent turbidity is plotted against time. Other conditions as in a), except that ● denotes 15.0 mM initial hemoglobin concentration.

plot of either the logarithm of the lag time (defined as the time for completion of half the total apparent turbidity change) or the logarithm of the pseudo-first order rate constant for the rapid phase against the logarithm of the initial deoxyHbS concentration showed that both depended on the same high power of the concentration, roughtly the fifteenth. Addition of IHP decreased the lag times and increased the rate constants by an order of magnitude.

In contrast, the depolymerization reaction (Fig. 2b) exhibited no such lag, and the rate constants for the two phases in the biphasic time course depend on a much lower power of the hemoglobin concentration, roughly the second. Addition of IHP decreased the rate of depolymerization, again by an order of magnitude. Gross hysteresis typical of multi-molecular aggregation processes (12) was found when the temperature was cycled between 2° and 30°, holding for

two hours at each temperature. Reproducible results of the type shown could only be obtained after equilibration of the samples at the initial temperature for at least 16 hours.

Quantitative interpretation of these results is hindered by a number of difficulties peculiar to deoxyHbS polymerization. The apparent turbidity of the sample contains both a non-negligible absorbance component and a true turbidity component due to scattering; the sample is highly non-ideal, and hence turbidity is no longer proportional to the weight-average molecular weight (13); the final aggregates are large with respect to the wavelength of the monitoring light. Nevertheless, certain qualitative conclusions can be drawn. The existence of a lag period and the extremely high power dependence on the deoxyHbS concentrations in the polymerization reaction appear to rule out models (7,8) involving simple linear condensation (Refs. 14 and 15; Fig. 1, reactions A, B, C and D) or stacking of hexameric discs (Ref. 9; Fig. 1, reactions E and G). Such models would generate at most second or sixth power dependence, respectively. Nucleated helical polymerization models (16) are capable of generating the observed power dependence, but require the final aggregates to have roughly 30 deoxyHbS molecules per turn of the polymerization helix. This is incompatible with electron microscope observations (9,17). It appears that the minimal model consistent with these results is one which involves rate-limiting formation of nuclei containing at least fifteen deoxyHbS molecules, followed by rapid polymerization, either of the nuclei themselves, or by addition of individual molecules to the nuclei. This highly concerted polymerization is consistent with equilibrium ultracentrifuge studies (18,19), where no significant concentrations of intermediate polymers could be detected. The effect of IHP on the polymerization and depolymerization rates is consistent with the facilitation of gelation in equilibrium experiments by other organic phosphates (19).

Closely related studies have recently been reported by Hofrichter, Ross and Eaton (20), using microcalorimetry and linear birefringence to monitor poly-

merization and depolymerization. They have interpreted the large concentration, temperature, and time-dependence as arising from a nucleation-controlled polymerization (20,21). Malfa and Steinhardt (22) have followed the polymerization using viscometry. In all three techniques, the time course of polymerization exhibits a marked lag followed by a rapid phase. However, turbidimetry, microcalorimetry, birefringence and viscometry are likely to be sensitive to different stages of the polymerization reaction. The relationship between the results obtained by these varied techniques must await further study.

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