

Solidity of sickle hemoglobin gels: relevance to pathophysiology of sickling disorders

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■ The physical properties of hemoglobin (Hb) S gels formed under conditions relevant to in vivo conditions have been characterized using viscometry. Mixed Hb SF and Hb AS gels of hemoglobin concentrations and compositions found in patients with Hb S-HPFH (mild sickle-cell disease) and Hb AS (sickle-cell trait) have been compared to those of pure Hb S. The results may explain why red cells containing gels occlude the microcirculation and/or undergo hemolysis in patients with sickle-cell anemia, why patients with Hb S-HPFH are mildly affected with red-cell sickling, and why individuals with Hb AS have no clinical manifestations of the Hb abnormality.

□ INDEX TERMS: ANEMIA, SICKLE CELL; HEMOGLOBIN, SICKLE; SICKLE CELL TRAIT □ CLEVE CLIN J MED 1989; 56:793-800

HEMOGLOBIN S (Hb S) differs from hemoglobin A (Hb A) by the substitution of a neutral (valine) for an acidic (glutamic acid) amino acid at the β_6 position. When deoxygenation occurs this substitution is critical for the formation of nuclei, polymers, long fibers, and gels. These in turn decrease red cell deformability, distort the red cell, and lead to hemolysis or vaso-occlusion of the microcirculation. The amount of intracellular polymer has been correlated with both hemolysis and clinical severity of the different sickling syndromes, supporting the hypothesis that the intracellular polymerization of Hb S is the primary determinant of severity in sickle hemoglobinopathies.¹

Studies in our laboratory of the rheologic behaviors of gels formed from pure Hb S and from mixtures of S and

A Hb or S and F Hb have shown that equivalent amounts of polymers have different physical properties (solidity, viscoelasticity) depending on composition and history of the gel, leading to the conclusion that not only polymer mass but also its physical properties dictate the fate of the red cell.²⁻⁴

This paper presents the physical properties of pure Hb S and mixed Hb SA and SF gels formed under conditions that are close to physiologic and compares the properties of gels that are formed in patients with Hb S-HPFH (mild disease) and sickle trait Hb AS (no disease) with those of Hb SS (severe disease). An overview of Hb S gelation is first presented.

Deoxyhemoglobin S polymerization can be followed kinetically using a variety of physical techniques such as viscosity, light scattering, turbidity, and nuclear magnetic resonance. Once the reaction is initiated, usually by temperature jump but also by rapid deoxygenation, a phase follows during which no change occurs in the signal being monitored. This delay (or lag, when monitored by viscometry) phase is extremely dependent on the concentration of deoxyhemoglobin S and temperature. At 0°C, deoxyhemoglobin S molecules are mono-

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merically dispersed when the concentration is <30 g/dL; whereas at 37°C , polymerization occurs and results in a deoxyhemoglobin S solubility of 16 g/dL.⁵ The existence of the delay time is evidence for a nucleation-controlled polymerization reaction.

After the formation of a small aggregate of hemoglobin molecules known as the "critical nucleus," further polymerization occurs at an explosive, autocatalytic rate that is signaled by an exponential increase in the physical property being monitored. This growth phase is then followed by the alignment of polymers and growth of polymer domains. The existence of a delay time, which is directly proportional to the 30th to 50th power of the hemoglobin concentration, forms the basis of the kinetic model of sickling in the microcirculation. This states that the probability that Hb S will polymerize in a cell as it becomes deoxygenated during passage through the microcirculation will be determined by the delay time of the Hb S (dependent on the hemoglobin concentration and composition, oxygen saturation, and shear history) relative to the transit time.⁵ Many therapeutic interventions have been directed toward decreasing the concentration of deoxyhemoglobin S intracellularly to increase the delay time.

Since the early 1950s, sickling has been known to be inhibited by the admixture of Hb A, F, and C with Hb S; Hb F more so than Hb A or Hb C. When Hb A is $>50\%$ of the hemoglobin in the red cell, as is the case in individuals with sickle trait (Hb AS; Hb A:S = 60%:40%), no clinical manifestations occur under most circumstances. The dilution of Hb S by Hb A prolongs the delay time 10^6 -fold compared to that of pure Hb S samples of the same total hemoglobin concentration (measured by turbidity).⁶ This has led some investigators⁶⁻⁸ to conclude that the goal of a "cure" in sickle-cell anemia (Hb SS) would be achieved if the delay time could be prolonged by this factor (equivalent to a solubility ratio of 1.5). Patients with Hb S- β^+ Thal (Hb S:A = 80%:20%) have hemolysis and vaso-occlusive episodes that are usually less severe than those with Hb SS, but the intracellular concentration of Hb S is sufficiently high to polymerize under oxygen saturations present in the microcirculation.¹ Delay times are prolonged relative to those of Hb SS of the same concentration by a factor of 10^2 (corresponding to a solubility ratio of 1.2), suggesting this degree of lengthening of delay times is a threshold for obtaining a therapeutic effect.

The presence of Hb F has a much greater solubilizing effect on Hb S than does Hb A. Patients with Hb S-HPFH who have 25% Hb F present in a pancellular distribution have minimal clinical manifestations of sick-

ling. The presence of 30% Hb F prolongs the delay time by a factor of 10^4 when compared to Hb S at the same concentration and temperature.⁶ Any agent that could increase the delay time by this amount (equivalent to a solubility ratio of 1.3) should produce a major therapeutic effect. Because of these clinical studies and supporting solutions studies, much attention has been focused on attempts to treat sickle-cell disease by increasing Hb F levels using pharmacologic means.⁹

The structure of the Hb S polymer is known to 1.5-nm resolution from electron microscopy and image reconstruction studies of the gels¹⁰⁻¹² and from x-ray diffraction studies of the deoxyhemoglobin S crystal.¹³⁻¹⁵ Several higher-order structures of Hb S polymers exist (tactoids, spherulitic domains, needle-like crystals, microfibrils) and reflect the degree of alignment, polymer lengths, and concentration of the gel.¹⁶⁻¹⁹ From knowledge of the molecular contacts in the Hb S fibers (polymers), the incorporation of Hb A, Hb F, and Hb SF hybrid would be expected to inhibit lateral contacts, leading to looser fibers with altered structures. The incorporation of Hb SA hybrid would not be expected to change the Hb S polymer since only one β^6 -val/hemoglobin molecule is involved in a lateral contact between hemoglobin molecules.

The polymerization of Hb S is affected by different and competing mechanisms in the presence of both Hb A and Hb F. First, the solubility of Hb S is increased by the presence of Hb A or Hb F, by Hb F to a greater extent than Hb A. This prolongs the lag phases at a given total hemoglobin concentration, in proportion to the amount of Hb A or F present in the mixtures, and changes the kinetics of nucleation with potential alterations in structure of the polymer domains. Second, the tendency for Hb A or Hb F to copolymerize with Hb S would be expected to alter the structure and solidity of the Hb S-type polymer. Since the γ chain of Hb F differs from the β chain of Hb S by 39 amino-acid substitutions, incorporation of Hb F would be expected to have marked effects on the polymer properties. Third, the presence of any hemoglobin (or any protein with similar configuration) in the highly concentrated solutions acts to increase the activity of Hb S through excluded volume effects due to the physical crowding of molecules in solution. This enhances polymerization. The physical properties of mixed hemoglobin gels reflect the relative contributions of these interactions.

Rheologic properties depend upon and reflect the structure of the gels. Although a few studies of pure Hb S gels have been published,²⁰⁻²³ these were done under limited conditions of hemoglobin concentration and

temperature that were far from physiologic, and no studies of mixed hemoglobin gels have (to our knowledge) been reported. The following experiments were undertaken using concentrations, hemoglobin compositions, and temperatures relevant to those found in vivo to identify factors responsible for gel physical properties.

METHODS

The detailed methods have been published elsewhere.²⁻⁴ Purified stripped solutions of Hb S, A, and F, dialyzed against 0.15-M potassium phosphate buffer, were deoxygenated by hydrated N₂ and Na₂S₂O₄ at 0°C to a pO₂ = 0 torr. Mean pH was 6.85; total hemoglobin concentration and composition were relevant to those in red cells in vivo. Hb S and A, and S and F, were mixed prior to deoxygenation to permit the formation of hybridized tetramers ($\alpha_2\beta^S\beta^A$, $\alpha_2\beta^S\gamma$). Samples were introduced under anaerobic conditions to the sample cup of a Wells-Brookfield HBT Digital microviscometer maintained at 37°C by a circulating water bath. The temperature jump initiated the sol-gel transformation. The viscometer was turned on at a shear rate of 38.4 sec⁻¹ and viscosity monitored until it increased fivefold (from 10 cps to 51.2 cps), signaling the end of the lag phase and the onset of polymerization. The viscometer was then stopped and the gel annealed (set, strengthened) in the absence of shear for 30 minutes before determination of gel rheologic properties by stress relaxation.

A target stress of 1100 dynes/cm² was imposed on the gels; thereafter, the stress held by the gel was followed over time. Solid gels held the imposed stress indefinitely and were characterized by their yield temperatures (the temperature at which stress suddenly decreased due to melting of the gel, obtained by lowering the temperature of the water bath; the lower the required temperature, the more solid the gel). The viscoelastic gels did not hold the target stress. These gels were characterized by the stresses at which they broke (yield stress) and the final stress attained (equilibrium stress).

Gels were ultracentrifuged to separate supernatant (individual hemoglobin molecules) and pellet (polymerized hemoglobin and trapped supernatant). The volume, concentration, and composition of each phase were measured, and the solubility of Hb S, polymer mass, polymer fraction, and polymer composition were calculated.

To control for the increased chemical activity of Hb S due to the highly concentrated solutions, samples were prepared using Hb S and deoxygenated bovine serum al-

bumin (BSA). BSA acts only to increase the chemical activity of Hb S and does not change Hb S solubility or copolymerize with Hb S.²⁴

The relative solidity of Hb SA and SF gels and gels formed from Hb S-BSA mixtures was obtained by dividing the yield temperature or yield stress of the mixed Hb gels by that of pure Hb S of the same polymer mass, polymer fraction, or lag time. The relative solidities were then averaged for the given mole fraction of Hb S (X_S) in the initial mixtures. The relative lag times were the ratio of the log lag times of the mixed Hb SA or Hb SF gels at a given X_S to the log lag time for pure Hb S at a given initial total hemoglobin concentration and temperature.

RESULTS

Pure Hb S gels

Gels resulting from Hb S solutions of concentrations ranging from 20.4 to 32.9 g/dL have been characterized after annealing for 30 minutes at 37°C. Lag times of the samples ≥ 28.1 g/dL were too short to resolve. Lag times of the less concentrated runs were dependent on the 18th power of the hemoglobin concentration. This lower concentration dependence of the lag time on hemoglobin concentration in our system compared to that determined by turbidity presumably reflects the known effect of shear to shorten the lag times.²⁵ The solubility of Hb S was 16.1 g/dL at 37°C, and was independent of the initial total hemoglobin concentration. Gels resulting from pure Hb S samples of initial total hemoglobin concentration ranging from 22.3 to 32.9 g/dL (polymer fractions of 0.38 to 0.66) held the imposed stress of 1100 dynes/cm² indefinitely (solid behavior) and broke suddenly at the yield temperature. Those of initial total hemoglobin concentrations of 20.4 to 22.0 g/dL (polymer fractions of 0.28 to 0.37) were viscoelastic and did not hold the imposed stress. The yield and equilibrium stresses and inverse yield temperatures were correlated with initial hemoglobin concentrations, polymer mass, and polymer fraction. Shear (stirring) had a pronounced effect on gel behavior. When gels were sheared during the polymerization process at 38.4 sec⁻¹ (comparable to shear rates in the arteries and arteriolar circulation), behavior was less solid (yield stress of 16 dynes/cm²) than the same sample not exposed to shear before the annealing phase (yield stress of 79 dynes/cm²). Shearing at 38.4 sec⁻¹ at the yield temperature for 5 minutes converted the gels into viscous solutions (stress decreased to 0 dynes/cm² when the viscometer was stopped).

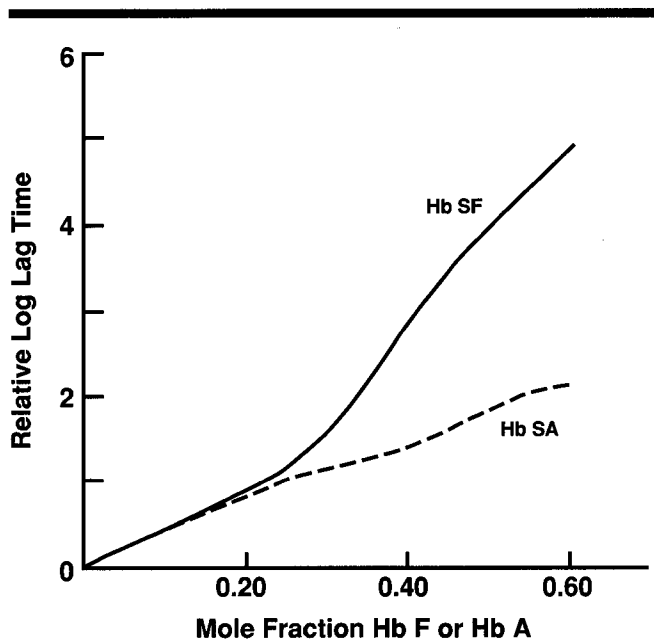


FIGURE 1. Effect of Hb A and F on the lag times. The log lag times of Hb SA and Hb SF mixtures relative to log lag time of pure Hb S are plotted ν the mole fraction of Hb A and F at constant total hemoglobin concentration.

Mixed Hb SA and SF gels

Gels resulting from mixtures of initial total hemoglobin concentrations 22.8 to 35.3 g/dL, and X_S ranging from 0.8 to 0.3, have been characterized after annealing for 30 minutes at 37°C. Lag times were related to hemoglobin concentration and composition of the initial mixture. The effects of Hb A and Hb F on the lag times at 37°C are drawn in Figure 1 where the log lag times relative to those of pure Hb S of the same total hemoglobin concentration are drawn for different amounts of Hb A or Hb F in the initial mixture. As X_S decreases in the initial mixture, the relative lag times increase compared to those of pure Hb S, SF mixtures to a greater extent than those of Hb SA. At $X_S = 0.7$, the lag times are increased 30-fold in the Hb SF mixtures (relevant to Hb S-HPFH); at $X_S = 0.4$, lag times were increased 180-fold in the Hb SA mixtures (relevant to Hb AS) compared to those of pure Hb S. These relative log lag times are three orders of magnitude lower than those reported for Hb SA and Hb SF mixtures monitored by turbidity,⁶ presumably reflecting the effect of shear on the lag times.

The hemoglobin solubility, determined at equi-

TABLE 1
MIXED Hb GELS: EQUILIBRIUM SOLUBILITY AND POLYMER COMPOSITION

X_S	C_0	[S]	C_s	[S] _s	f_s	f_A	f_F	f_{BSA}
Hb SA:								
0.8	30.3	23.0	17.9	12.2	0.83	0.17	—	—
0.6	31.0	18.0	23.0	11.7	0.69	0.31	—	—
0.4	30.3	12.1	25.8	8.3	0.50	0.50	—	—
Hb SF:								
0.8	28.0	22.1	19.8	14.7	0.86	—	0.14	—
0.6	31.6	21.5	25.0	14.0	0.76	—	0.24	—
0.4	31.8	12.7	30.0	11.1	0.65	—	0.35	—
Hb S-BSA								
0.7	29.5	20.0	22.2	11.0	1.00	—	—	0
0.5	33.5	17.0	25.9	5.8	1.00	—	—	0

X_S = mole fraction of Hb S in initial mixture; C_0 = initial total hemoglobin concentration (g/dL); [S] = Hb S concentration of initial sample (g/dL); C_s = supernatant concentration after ultracentrifugation of gel (g/dL); [S]_s = Hb S concentration in supernatant (g/dL); f_s, f_A, f_F, f_{BSA} = fraction of polymer that is Hb S, A, or F or bovine serum albumin, respectively.

TABLE 2
SOLIDITY OF Hb S, SA, AND SF GELS: SIMILAR INITIAL TOTAL HEMOGLOBIN CONCENTRATIONS

C_0 (g/dL)	X_S	Yield temp (°C)	Polymer mass (mg)
Hb S			
29.4	1.0	<3.5	165
Hb SA			
30.3	0.8	5.8	167
31.0	0.6	12.0	120
29.6	0.4	23.5	71
Hb SF			
29.4	0.7	19.0	138
29.4	0.5	—*	85

C_0 = initial total hemoglobin concentration; X_S = mole fraction of Hb S in initial sample.

*(Yield and equilibrium stresses = 71 and 2 dynes/cm², respectively).

librium by ultracentrifugation of gels and measurement of the supernatant concentration and composition, was independent of the initial total hemoglobin concentration and increased as X_S decreased, more so with Hb F than with Hb A (Table 1). Both Hb A and Hb F were incorporated into the polymer when present in high proportions in the initial mixtures. When $X_A = 0.6$, 50% of the polymer was Hb A; when $X_F = 0.6$, 35% of the polymer was Hb F (Table 1).

Solidity was related to initial hemoglobin concentration and X_S in the sample as well as polymer mass and polymer fraction. When compared to pure Hb S gels on the basis of initial total hemoglobin concentrations, Hb SA gels were less solid than those of pure Hb S, and Hb

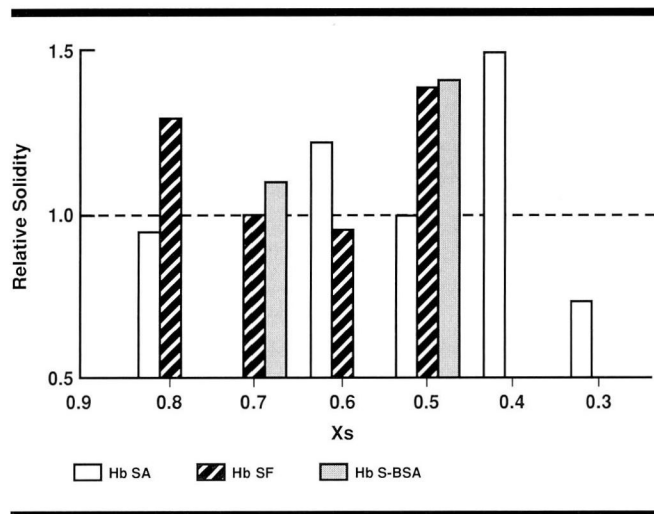


FIGURE 2. Effect of Hb A and F and BSA on solidity at constant polymer mass. The solidities of Hb SA and SF and Hb S-BSA gels relative to those of pure Hb S gels of the same polymer mass are plotted v the mole fraction of Hb S in the initial mixture.

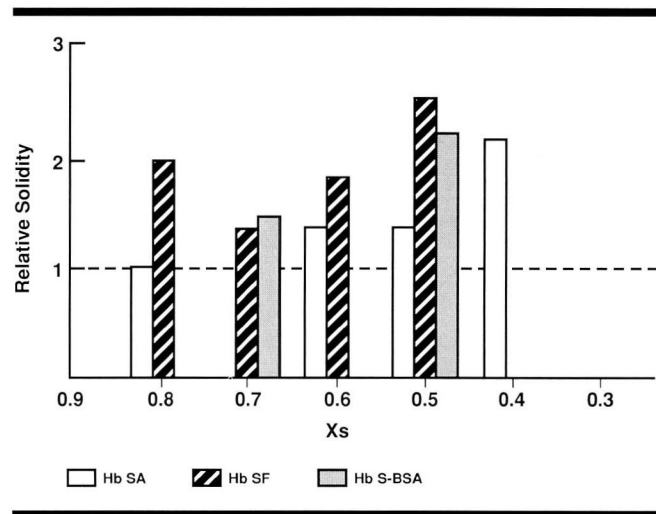


FIGURE 3. Effect of Hb A and F and BSA on solidity at constant polymer fraction. The solidities of Hb SA and SF and Hb S-BSA gels relative to those of pure Hb S gels of the same polymer fraction are plotted v the mole fraction of Hb S in the initial mixture.

SF gels were the least solid. This pattern reflects less polymer mass in the mixed gels (Table 2). When expressed on the basis of comparable polymer mass (Figure 2), Hb SA and SF gels were similar to or more solid than those of Hb S when X_S decreased from 0.8 to 0.6, but an apparently anomalous finding was observed as X_S decreased further. Instead of becoming less solid, as expected from the effects of incorporation of large amounts of Hb A and F into the polymer, gels became more solid than those of pure Hb S when $X_F = 0.5$ and when $X_A = 0.6$. When expressed on the basis of similar polymer fractions or lag times (Figures 3 and 4), Hb SA and SF gels became progressively more solid than those of pure Hb S as X_S decreased from 0.8 to 0.4.

DISCUSSION

The conditions under which sickle hemoglobin gels have been characterized are similar to those in vivo in terms of hemoglobin concentration and composition, osmolality, temperature, pH, and shear history. The complete oxygen desaturation and annealing times of 30 minutes are extremes that would be most unlikely to be present in patients. However, solutions of Hb S develop solid behavior within seconds after the onset of polymerization,²⁰ and the calculated polymer mass of the densest red cells includes enough polymer to have solid behavior

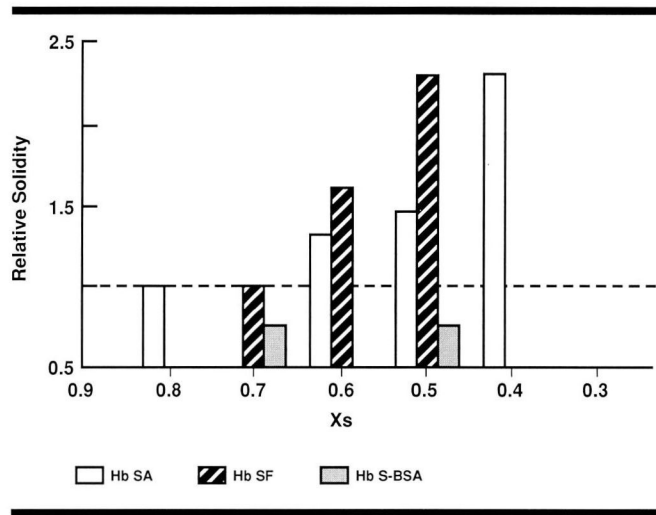


FIGURE 4. Effect of Hb A and F and BSA on solidity at constant lag times. The solidities of Hb SA and SF and Hb S-BSA gels relative to those of pure Hb S gels of the same lag time are plotted v the mole fraction of Hb S in the initial mixture.

at oxygen saturations present in the capillaries. (An SS red cell of MCHC = 40 g/dL has a polymer fraction of 0.24 and polymer mass of 96 mg at an O_2 saturation of 70%.⁹) We are of the opinion that the behaviors of the

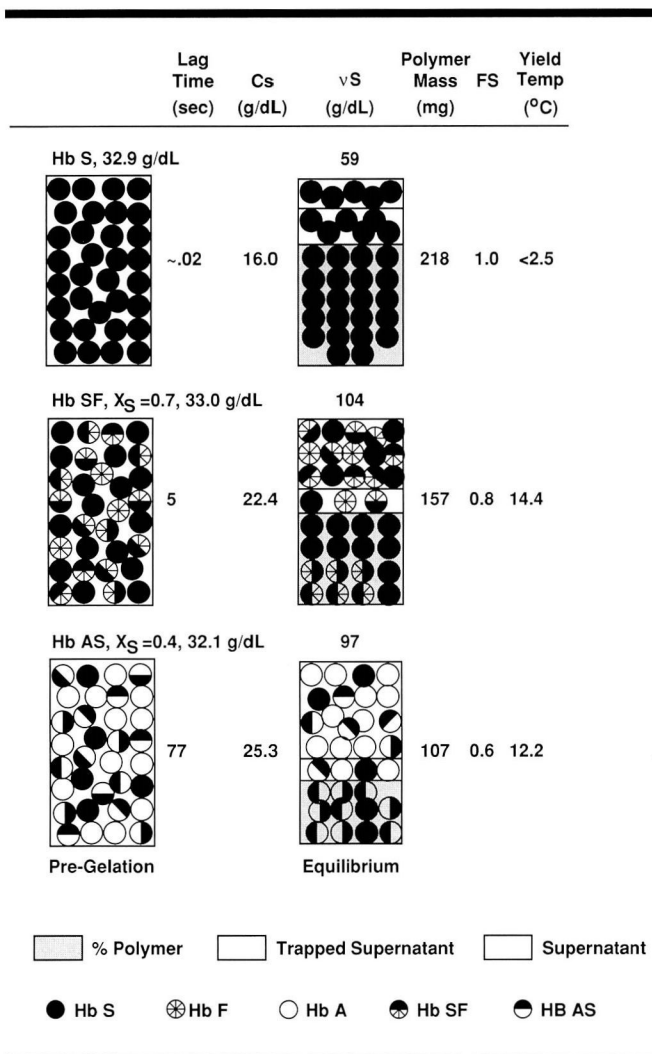


FIGURE 5. Hb S, Hb SA, and Hb SF mixtures of comparable initial total hemoglobin concentrations and of X_S similar to that of Hb SS, Hb AS, and Hb S-HPFH at time 0 (pre-gelation) and at equilibrium. Numbers of hemoglobin molecules in proportions and types are drawn in the phases as determined experimentally. vS is the chemical activity of HbS. FS is the fraction of polymer that is HbS.

gels formed of Hb S solutions and characterized in vitro can be extrapolated to gels that would occur in red cells in patients with Hb SS and other sickling disorders.

Pure Hb S gels formed from initial total hemoglobin concentrations approaching those in erythrocytes of patients with Hb SS (where mean corpuscular hemoglobin concentrations range from 26 to 48 g/dL) held an imposed stress of 1100 dynes/cm² indefinitely. If gels of similar degrees of solidity formed intracellularly, they

could never be broken by shear forces transmitted across the membrane. (In vivo wall shear stress in most of the arteriolar circulation is ~50 dynes/cm².²⁶) If a stress of sufficient magnitude to disrupt the gel were to be imposed on the cell, hemolysis would occur. Membrane damage is present when red cells are exposed to shear stresses of 250 dynes/cm² in vitro, and erythrocytes hemolyze when stress levels are as low as 755 dynes/cm².²⁷

Mixed hemoglobin gels from initial total hemoglobin concentrations and compositions relevant to those found intracellularly in patients with Hb S-HPFH and Hb AS were also solid when studied under these conditions. As for the pure Hb S gels, the stresses held were an order of magnitude greater than those found in vivo, so that the gels would not be expected to be disrupted by in vivo shear forces. As X_S was decreased in the initial mixtures, an apparently anomalous behavior was seen in the Hb SA and SF gels. Instead of becoming less solid when X_S decreased (as is expected from incorporation of greater amounts of non-S Hb into the polymer), Hb SA and SF gels were more solid than gels of pure Hb S of comparable polymer mass when X_A = 0.6 and X_F = 0.5.

These behaviors can be interpreted in terms of the influence of increased Hb S chemical activity on gel solidity. The Hb S-BSA mixtures are more solid than pure Hb S gels of similar polymer mass or polymer fraction (Figures 2 and 3). In these studies, no albumin enters the polymer, polymer composition is 100% Hb S, the solubility of Hb S is not increased by BSA (confirmed by ultracentrifugation studies [Table I], and the fact that lag times are decreased compared to those of either Hb SA or SF mixtures of similar initial total concentrations and X_S), and Hb S chemical activity is one to two times that of pure Hb S. In the Hb SF studies, Hb S chemical activity is two to seven times that of pure Hb S, and in the Hb SA gels it is one to five times that amount. The greatest Hb S chemical activity is in the gels with the least X_S.

The results support the conclusion that the increased chemical activity of Hb S is a primary determinant of the gel physical properties and structure, and that this effect overrides the influences of the other interactions of Hb A and F with Hb S on gel properties (ie, incorporation of non-S Hb into the polymer and the influences of non-S Hb on nucleation kinetics with consequent changes in higher-order polymer structures).

The shear history of the gel was a major determinant of solidity in both pure Hb S and mixed hemoglobin samples. Gels that were sheared throughout the polymerization process at shear rates of 38.4 sec⁻¹ were much less solid than those not exposed to shear before the an-

nealing phase. This suggests that gels formed in the arteriolar circulation (where shear rates are $\sim 40 \text{ sec}^{-1}$) would be less solid than the same mass of gel formed in the venules where shear rates approach 0 sec^{-1} . The cells containing less solid gels would be expected to be more deformable and to pass through the microcirculation. However as the cell undergoes further deoxygenation in the venous circulation, polymer mass would increase and have more solid behavior. Formation of gel in the venous circulation would be deleterious if not dispersed during cell oxygenation in the lungs and upon exposure to the shear forces of the arteries and arterioles. As the cell approached the capillaries, deoxygenation would increase polymer mass and lead to further decreases in cell deformability, with consequent vaso-occlusion and hemolysis. In Hb SS, approximately 20% of the erythrocytes, in Hb S-HPFH, $\sim 3\%$,⁹ and in Hb AS, none of the red cells contain gel while in the venous circulation.

The following example compares the kinetic and equilibrium properties of gels formed of solutions that would be present in red cells of patients with Hb SS, Hb S-HPFH, and Hb AS. *Figure 5* (left panel) shows the initial samples under completely deoxygenated conditions at 0°C , each symbol representing one pure hemoglobin molecule, with the hybridized Hb SA or Hb SF hemoglobin molecules drawn in the proportions known to occur under dilute conditions (and assumed to occur in more concentrated solutions). A temperature jump to 37°C initiates the nucleation process (lag time is shown in seconds). The sample in the right panel is drawn at equilibrium after ultracentrifugation of the gel separates the supernatant and pellet phases. For the mixed hemoglobin gels, the composition of the polymer is drawn using the appropriate symbols. The solidity of the gels is noted as the yield temperature. What is evident is that under these extreme conditions, the pure Hb S sample of 32.9 g/dL has a lag time too short to resolve

(estimated to be ~ 0.02 seconds), the Hb SF sample of 33.0 g/dL , $X_S = 0.7$, has a lag time of 5 seconds, and that of the Hb AS sample of 32.1 g/dL , $X_S = 0.4$, is 77 seconds. The amount of polymer formed is very different in the three samples, also a reflection of the effects of Hb F and A on Hb S solubility. Polymer fractions are 0.66, 0.48, and 0.33, respectively, for the HbS, HbSF, and Hb AS gels. The pure Hb S gel is extremely solid (yield temperature $< 2.5^\circ\text{C}$); the Hb SF and SA gels are also solid but less so (yield temperatures of 14.4 and 12.2°C , respectively).

These observations suggest that under equilibrium conditions where polymer fractions are 0.3–0.66, pure and mixed Hb S gels, although influenced by shear history, are extremely solid and can never be disrupted without causing membrane damage and hemolysis. They confirm that it is the marked increase in delay times occasioned by the dilution of Hb S by Hb A and the increase in Hb S solubility that protects individuals with sickle trait from clinical manifestations.

In planning therapeutic manipulations for patients with Hb SS, the following points can be made: Pharmacologic stimulation of Hb F in a pancellular distribution to levels of 30% of the total hemoglobin would ameliorate clinical manifestations by prolonging the delay times. Even greater increases in Hb F may be necessary to insure that no polymerization occurs during the 1 second that a red cell takes to traverse the microcirculation, since once Hb SF gels are formed, they are just as solid as those of pure Hb S of similar mass. The marked effect of increased Hb S chemical activity on gel properties suggests that manipulations designed to decrease the intracellular hemoglobin concentrations and concentrations of polymerizable hemoglobin S would be more efficacious than those that would increase Hb S oxygen affinity or inhibit interpolymer contacts by chemical means.

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