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Interaction Between Human Hemoglobin Variants and Hemoglobin S (40031)

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Data from optical and ultrastructural studies have offered considerable insight into the location of contacts between hemoglobin molecules, and additional information concerning these intermolecular bonds has come from studies of factors which promote or inhibit gelation of Hb S. Of particular interest are the results of studies of the effects of specific amino acid substitutions at the intermolecular surface.

Several hemoglobin variants, such as the β chain variants Deer-Lodge (2 His → Arg), C (6 Glu → Lys), C-Harlem (6 Glu → Val, 73 Asp → Asn), G-Makassar (6 Glu → Ala), Leiden (6 or 7 Glu → O), J-Baltimore (16 Gly → Asp), N-Baltimore (95 Lys → Glu), O-Arab (121 Glu → Lys), D-Los Angeles (121 Glu → Gln), and the α chain variants I-Philadelphia (16 Lys → Glu) and Memphis (23 Glu → Gln) have been studied with regard to their influence on the gelation and solubility of deoxy Hb S (1-9). The data have shown that variants influencing the gelation or the solubility of deoxy Hb S or both have a substitution of one of the following residues: Asp 73β, Glu 121β, Lys 16α, and Glu 23α (4, 10-12). The substitution of a specific amino acid residue can weaken the interaction between the molecules (e.g. the Asp → Asn substitution at 73β) or strengthen the intermolecular contact (e.g. the Glu → Lys substitution at position 121β).

Recently we were able to study the effect of additional variants together with some variants already investigated, both in the deoxy- and oxyconformation; these include the three substitutions at position 121β, namely O-Arab (121 Glu → Lys), D-Los Angeles (121 Glu → Gln), Beograd (121 Glu → Val), and also E (β26 Glu → Lys), N-Baltimore (β95 Lys → Glu), I-Philadelphia (α16 Lys → Glu) and G-Georgia (α95 Pro → Leu). Hb C (β6 Glu → Lys) and Hb A were used as control.

Materials and Methods. Hemolysates were made from saline washed red cells. The hemoglobins O-Arab and C were obtained from patients homozygous for this variant, Hb Beograd from the patient with Hb Beograd β°-thalassemia (13) (with about 90% of the variant), and Hb N-Baltimore from a patient with a Hb N-β^{+}-thalassemia (with 80% Hb N); red cell hemolysates were used without further treatment. The hemoglobins D-Los Angeles, E, G-Georgia and I-Philadelphia were isolated from red cell hemolysates of heterozygotes by DEAE-cellulose or by DEAE-Sephadex chromatography (14, 15). Red cell hemolysates from patients with sickle cell anemia were used as a source of Hb S; the level of Hb F (F_{AD}) in these samples was 4% or less.

Cyanferri hemoglobins were prepared with threefold excess of $K_3Fe(CN)_6$ and fourfold excess of KCN in 0.1 *M* phosphate buffer of pH 6.8; Sephadex G-25 chromatography was used to remove excess ferricyanide and KCN. The concentration of Hb was calculated from the millimolar extinction coefficient and absorbance at 540 nm after the sample was converted into cyanferrihemoglobin (16). Solutions of hemoglobin (either oxy- or cyanferri-) were concentrated by vacuum dialysis against distilled water using specific dialysis membranes (collodion bags #100 for protein concentration, capacity 8 ml, MW 25000, Schleicher and Schull, Inc., Lot #221/423); their visible spectra were verified using a Perkin Elmer double beam spectrophotometer (Coleman model 124 D). Minimum gelling concentration determinations were carried out at 25° on mixtures of deoxy Hb S and either the deoxy- or the cyanferri-derivative of other variants using the method of Singer and Singer (1) as modified by Bookchin *et al.* (5).

Results. The data, presented in Fig. 1,

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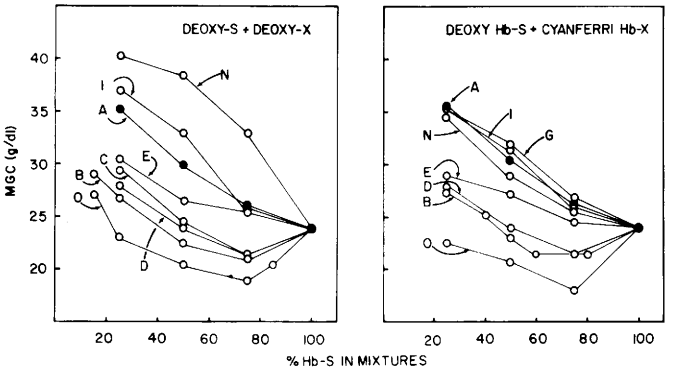

FIG. 1. The effect of increasing amounts of various β chain and α chain variants on the minimum gelling concentration (MGC) of deoxy Hb S. Left panel: oxy derivatives of the hemoglobins N-Baltimore, I-Philadelphia, A, E, C, D-Los Angeles, B (-Hb Beograd) and O-Arab mixed with oxy Hb S prior to deoxygenation; right panel: the cyanferri-derivatives of the same derivatives and of G-Georgia mixed with oxy Hb S prior to deoxygenation. Temperature 25°. The standard deviation for a mean gelling concentration of 24.40 was 1.38 with an error of the mean 0.61. (n = 5).

confirm and extend the data obtained by others (1-3). The strongest positive effect was observed for Hb O-Arab, followed by Hb Beograd, Hb D-Los Angeles, and Hb C. The effect of Hb E was much less pronounced and Hb I-Philadelphia and Hb G-Georgia affected the gelation of deoxy Hb S to a similar extent as Hb A. The effect of each of the variants was the same whether in the deoxy or oxy conformation, with the exception of Hb N-Baltimore. In the deoxy state, this variant exhibited a strongly negative effect on the gelation of deoxy Hb S but behaved like Hb A in the oxy (cyanferri-) state.

Discussion. Five of the eight variants studied have a Lys → Glu (N, I) or a Glu → Lys (C, E, O-Arab) substitution. Substitution of Lys at 95β by Glu greatly decreases intermolecular interaction, but a similar substitution at 16α has no effect. In contrast, a Glu → Lys substitution appears to increase intermolecular interaction but the effect is again greatly dependent upon the location of the substitution. It has been assumed that Glu 121β forms a hydrogen bond with Lys 17β of the same chain; the latter residue forms an intermolecular hydrogen bond with carbonyl oxygen of His 117β_2 of the neighboring molecule in the same strand (17). Thus, substitution of Glu 121β by either Val or Gln or Lys would weaken the bond between this residue in position 121 and Lys 17β, and would make Lys 17β more accessible for the formation of intermolecular hydrogen bonds (17). The difference in effect between Hb O-Arab, Hb Beograd or Hb D-Los Angeles on the gelation of deoxy Hb S may be explained by the formation of an additional intermolecular hydrogen bond when a lysyl residue is introduced in position 121β. Similarly, substitution of a lysyl residue in position 95β by a glutamyl residue may result in the loss of a bond that participates in intermolecular interaction of deoxy hemoglobin molecule thereby decreasing the gelling interaction. Such an effect is not apparent for lysyl residue in position 16α.

These results support X-ray data (17) and extend the correlation observed (2) between the clinical severity of a number of sickling variants and the effect of the variants on the gelation of Hb S in mixtures.

Summary. Seven abnormal hemoglobins were investigated for an effect on the gelation of Hb S in the deoxy- or oxy-conformation. A positive effect (decreased MGC) was associated with substitution of Glu β6, Glu β26 and Glu β121. No difference was observed between the deoxy- or oxy-hemoglobins except in the case of Hb N-Baltimore which, in the deoxy state, exhibits a strongly negative effect on gelation but behaves like Hb A in the oxy state.

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