Structure and Properties of Hemoglobin CHarlem, a Human Hemoglobin Variant with Amino Acid Substitutions in 2 Residues of the β-Polypeptide Chain*

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SUMMARY

A new sickling hemoglobin variant designated hemoglobin CHarlem (Hb Cn) migrated slightly anodally to the position of hemoglobin C on electrophoresis at pH 8.6, and has been shown to have the structure αt * βt Glu → Val, 73 Asp → Asn. Hemoglobin Cn comprised 40% of the hemoglobin in individuals of two generations of an American Negro family and is the first instance in which two amino acid substitutions in a single polypeptide chain have been defined in a human hemoglobin variant.

Hemoglobin CH comprised 40% of the hemoglobin in individuals of two generations of an American Negro family and is the first instance in which two amino acid substitutions in a single polypeptide chain have been defined in a human hemoglobin variant.

Hemoglobin Cn appeared not to differ significantly from Hb A in its oxygen equilibria, ultraviolet spectra of the oxy and deoxy forms, and titratable sulfhydryl groups. Probably by virtue of its βt Val substitution, Hb Cn shared with Hb S (αt * βt Glu → Val) the properties of erythrocyte sickling and relative insolubility and gelation of the deoxyhemoglobin. However, gelation experiments suggested that the 73 Asn substitution resulted in a decrease in the intermolecular interactions of deoxyhemoglobin Cn as compared with deoxyhemoglobin S, and implied conformational differences between the two deoxyhemoglobins.

More than 40 variants of human hemoglobin have been described which differ in primary structure from their normal counterparts by a single amino acid substitution in each of the like polypeptide chains (1). The present report is concerned with a new hemoglobin variant, designated hemoglobin CHarlem (Hb Cn), which is associated with erythrocyte sickling and which contains two amino acid substitutions in each β-polypeptide chain: its structure has been shown to be αt * βt Glu → Val, 73 Asp → Asn. This report includes a description of the studies (some of which were the subject of a preliminary account (2)) leading to this structural formulation and a comparison of some properties of Hb Cn and Hb S, particularly with reference to sickling.

EXPERIMENTAL PROCEDURE

Source of Hemoglobin CHarlem—The propositus was a 45-year-old Negro man referred by Miss Mary McKenna, senior chemist at Harlem Hospital, who observed erythrocyte sickling associated with a paper electrophoretic pattern of hemoglobins C and A and decreased solubility of the deoxyhemoglobin. Hemoglobin electrophoresis on starch gel at pH 8.6 (3) showed Hb A and a component migrating slightly anodal to the position of Hb C (Fig. 1). The hemolysate prepared by the method of Drabkin (4) contained 40% Hb Cn, 2.3% fetal Hb, and the remainder Hb A. The normal minor basic component, Hb A2, could not be distinguished in the presence of Hb Cn.

Hemoglobin CHarlem was found in similar proportions (40%) in six of the nine children of the propositus whose red cells also sickled in 2% sodium metabisulfite. These heterozygotes were clinically well. Further clinical studies will be described separately.

Studies of Primary Structure—Acid dissociation and recombination experiments were carried out by the method of Itano and Singer (6) with minor modifications. Hemoglobin Cn was isolated by DEAE-cellulose chromatography by the method of Huisman and Dozy (7) or by starch granule electrophoresis (8) in Veronal buffer, pH 8.6. Neither method resulted in separation of Hb Cn from Hb A2. The proportion of Hb A2 would be expected to double or triple (the usual proportion is 2 to 3%) during the isolation procedure. For comparative studies, Hb S was isolated by similar methods to remove most of the Hb F, which amounted to less than 2% following DEAE-cellulose chromatography. The β chains of Hb Cn were isolated by a modification of the method of Bucci and Fronticelli (9) in which HMB2 was added to the hemoglobin which had been dialyzed for 4 hours against 0.05 M phosphate buffer, pH 5.8, containing 0.05 mole of NaCl, and the αHMB and βHMB chains were subsequently separated by electrophoresis on starch granules in Veronal buffer at pH 8.6. Since Hb A2 does not readily dissociate into α and β...
chains under these conditions,1 appreciable contamination of Hb Cn with Hb A did not occur during isolation of β\textsuperscript{HMB} chains of Hb Cn. Globin was prepared from β\textsuperscript{HMM} chains of Hb Cn and Hb A by acid-acetone precipitation (10), washed with acetone, and lyophilized. Tryptic digestion was carried out for 90 min at pH 8.0 as described by Ingram (11) except that lyophilized globin rather than heat-denatured hemoglobin was digested in air rather than a nitrogen atmosphere. Peptide maps (combined paper electrophoresis and chromatography) were prepared by Baglioni's modification (12) of the method of Ingram (11) from the soluble tryptic digest of the β\textsuperscript{HMM}-globin. Tryptic digests were also made of aminoethylated β-globin by the method of Clegg, Naughton, and Weatherall (13) to examine the otherwise insoluble "core" peptides. The peptide maps were stained for arginine, histidine, tryptophan, and sulfur (14).

Peptidase at 40° for 4 hours (16) and for 24 hours. For the products and the resulting peptides, after elution and acid hydrolysis, the peptides were eluted with 6 N HCl from the paper hydrolyzed at 105° for 22 hours. Contents of the capillary tubes were analyzed directly (13) in a Beckman model 120B automatic amino acid analyzer.

Papain digestion was performed on β\textsuperscript{HMB}Tp I eluted from peptide maps with 10% acetic acid. Peptide, 2 to 3 μmoles (obtained from 10 to 12 peptide maps), was digested at 40° for 15 hours in 0.015% papain at pH 5.5 and at pH 4.5 by the methods of Hill, Swenson, and Schwartz (16).

Subtractive Edman degradation followed the method of Konigsberg and Hill (17), with the minor modifications of Smyth, Stein, and Moore (18).

Pepsin digestion of β\textsuperscript{HMB} Tp IX peptides eluted from peptide maps with 10% pyridine was performed at 25° for 20 hours in 0.01% pepsin (17). Peptide maps were made of the digestion products and the resulting peptides, after elution and acid hydrolysis, were analyzed as described above. One of these peptic peptide was also hydrolyzed enzymatically with leucine amino-peptidase\textsuperscript{a} at 40° for 4 hours (16) and for 24 hours. For the longer digestion, an additional amount of leucine aminopeptidase equal to one-third of the initial amount was added to the digestion mixture after 12 hours.

Other Studies—Visible and ultraviolet spectra of oxy- and deoxyhemoglobin Cn were recorded on a Cary model 14R recording spectrophotometer. For preliminary observations of oxygen equilibria at 10°, the spectrophotometric technique of Allen, Guthie, and Wyman (19) with minor modification was employed. Reactive sulphydryl groups were titrated with HMB according to Benech and Benech (20). Sedimentation velocities were determined on the Spinco model E analytical ultracentrifuge at 10° with the use of an An-D rotor at 50,700 rpm, single sector cells, schlieren optics, Kodak 1D spectrophotometric plates, and a Corning 2-61 red filter; hemoglobin solutions were approximately 0.5 g/100 ml in 0.1 M sodium phosphate, pH 7.2.

Solubility—Hemoglobin S and Cn isolated by starch granule electrophoresis as described above were dialyzed against distilled water at 4°. Solubility determinations were carried out according to the method of Itano (21) with these modifications: phosphate buffers of varying ionic strength prepared from a single stock solution of 2.8 M potassium phosphate at pH 6.8 were used; the total solution for each determination was 5 ml; precipitates were separated by both centrifugation and filtration through Whatman No. 3 filter paper; and hemoglobin concentrations were determined after conversion to cyanmethemoglobin with 64,500 as the molecular weight of hemoglobin. Duplicate determinations of solubility were carried out four times for deoxyhemoglobin Cn, three times for deoxyhemoglobin S, and once for oxyhemoglobin Cn and S.

Gelation—Solutions of hemoglobin Cn and S, isolated as described above, were concentrated by ultrafiltration and were dialyzed against 0.15 M potassium phosphate at pH 7.35 before gelation experiments. Gelling studies at 25° were carried out by the method of Singer and Singer (22) with the following modifications: a flow of nitrogen gas, rather than CO2, was used for deoxygenation; gelation experiments with the isolated hemoglobins were performed on 0.5-ml aliquots in 10-ml Erlenmeyer flasks; and hemoglobin concentrations were determined as cyanmethemoglobin in a Beckman DU spectrophotometer. Fresh hemoglobin samples were diluted with distilled water to deter-
mine minimum gelling concentrations. Sometimes the concentration of a nongelling solution was increased very slowly by evaporation in the testing apparatus; under the flow of water equilibrated nitrogen, the small increase (up to 2 to 3 g/100 ml after 2 hours) observed in the concentration of the hemoglobin solution probably accounted for eventual gelation of samples which were very viscous after 60 to 90 min of deoxygenation. The proportions obtained by mixing solutions of isolated hemoglobins were verified by quantitative elution after starch block electrophoresis and found to vary from the intended proportions within ±4% of the total hemoglobin. No difference in results of minimum gelling concentrations were observed in hemolysates prepared by freeze-thaw of erythrocytes followed by exposure to toluene and centrifugation.

**RESULTS**

**Structure of Hemoglobin C_H**—At pH 4.7, a single “new” hemoglobin species was observed in recombination experiments with the use of Hb C_H and Hb I, which contains abnormal \( \alpha \) chains, and no new species were noted in experiments with Hb C_E and Hb C, which contains abnormal \( \beta \) chains (Fig. 2). These results were consistent with the observations of Itano and Robinson (23) on the hybridization of hemoglobins C and I in which the resulting new species (Hb A and the doubly abnormal hemoglobin \( \alpha^C \beta^C \)) had the same electrophoretic mobility and were observed as a single electrophoretic component. Thus the results of recombination studies indicated that the \( \beta \) chains of Hb C_E were abnormal.

Preliminary peptide maps of the tryptic digest of electrophoretically isolated Hb C_H were initially thought (24) to show findings closely resembling those of Hb Georgetown (25). However, when tryptic digests of isolated \( \beta^C \) chains were studied, the peptide maps contained \( \beta \) chain peptides differing from those reported for Hb Georgetown. Some of the initial difficulties may have been related to contamination of Hb C_H with Hb A_E; the isolated \( \beta \) chains were essentially free of contamination with Hb A_E or with \( \delta \) chains.

Comparison of peptide maps of the soluble tryptic peptides of isolated \( \beta^C \) chains with those prepared from isolated normal \( \beta \) chains (Fig. 3) showed the following differences: (a) \( \beta^C \)Tp I (numbering system of Gerald and Ingram (26)) was missing, and a new peptide with a greater \( R_f \) moved further toward the cathode.
TABLE II  
Molar ratios of amino acid residues after acid hydrolysis of \( \beta \)-tryptic Peptides IX and VIII-IX from hemoglobin A and from hemoglobin Csa

<table>
<thead>
<tr>
<th>Residues</th>
<th>( \beta^{\text{IX}} )</th>
<th>( \beta^{\text{IX}} )</th>
<th>( \beta^{\text{IX}} )</th>
<th>( \beta^{\text{IX}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>1.0</td>
<td>1.0</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>His</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Asp</td>
<td>2.9</td>
<td>3.3</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Ser</td>
<td>1.2</td>
<td>1.0</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Gly</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Ala</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Val</td>
<td>1.1</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Leu</td>
<td>3.7</td>
<td>4.0</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Phe</td>
<td>0.9</td>
<td>0.9</td>
<td>1.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

in the position of \( \beta^6 \) and \( \beta^7 \), the abnormal \( \beta^6 \) peptide was digested with papain (Fig. 4). Digestion at pH 5.5 yielded predominantly Peptides pap 1 and pap 2 and free lysine. At pH 4.5, Peptide pap 3 and free leucine were also prominent. Although free glutamic acid was found, a peptide containing only threonine, proline, and valine, as found by Hill, Swenson, and Schwartz in the papain digest of \( \beta^6 \) (16), was not recovered. The neutral zone on the peptide map of the papain digest contained contaminating peptides (not present in the original peptide), thought to be products of papain autodigestion, which could have masked a neutral Thr-Pro-Val peptide if it were present.

The peptide consisting of (Thr, Pro, Val, Glu) was eluted from the peptide map of a subsequent papain digest of \( \beta^6 \) and Edman degradation was performed in three stages yielding the following molar ratios (glutamic acid = 1). The yields, from each step, based on the aliquots taken for amino acid analyses, are shown in parentheses.

Stage 1 (99%): Thr, trace; Pro, 1.1; Val, 0.9; Glu, 1.0.  
Stage 2 (96%): Thr, trace; Pro, trace; Val, 0.7; Glu, 1.0.  
Stage 3 (27%): Thr, 0.0; Pro, 0.0; Val, 0.0; Glu, 1.0.  

These results established the substitution of valyl for glutamyl at the \( \beta^6 \) position of Hb Csa.

Studies of \( \beta^6 \) and \( \beta^7 \) Peptides VIII-IX—\( \beta^6 \) VIII is free lysine from position 66 which is variably cleaved during tryptic digestion from the \( \beta^6 \) valyl residue of \( \beta^6 \) IX, resulting in the two soluble tryptic Peptides \( \beta^6 \) IX and \( \beta^7 \) VIII-IX (26) of the sequences shown in Scheme 2 (27). The amino acid composition of the acid hydrolysates of the other two different peptides was the same as the composition of their normal counterparts, \( \beta^6 \) IX and \( \beta^7 \) VIII-IX (Table II), but the electrophoretic mobilities (Fig. 3) indicated that each \( \beta^6 \) peptide had a greater positive charge than the corresponding peptide from hemoglobin A. Such findings could result from the replacement of 1 of the aspartyl residues by asparaginyl since acid hydrolysis readily cleaves the amid group of asparagine (28) and aspartic acid is found on subsequent amino acid analysis. Findings similar to these were encountered by Lehmann, Beale, and Boi-Doku (29) in Hb G Accra of \( \beta^6 \) chain (pep 2A, Fig. 6) corresponded to residues 71 to 75 of the \( \beta^6 \) chain, which on the peptide map of \( \beta^7 \) IX appears the same as the position of \( \beta^7 \) IX.

To define the abnormality in \( \beta^6 \) IX, this peptide and \( \beta^7 \) IX were digested with trypsin. Peptide maps of the resulting digests (Fig. 5) revealed that an anodally migrating peptide from \( \beta^6 \) IX, which on analysis after acid hydrolysis (pep 2A, Fig. 6) corresponded to residues 71 to 75 of the \( \beta^6 \) chain, was missing on the peptide map of the \( \beta^6 \) IX digest. The latter contained a new peptide which migrated in the neutral zone, and which represented residues 71 to 75 (71 to 74 of some digests) of the \( \beta^6 \) chain (pep 2C and 2C, Fig. 6), thus suggesting that the aspartyl residue at \( \beta^6 \) was replaced by asparaginyl in Hb Csa.

To confirm this substitution, the peptic Peptide 2C from \( \beta^6 \) IX was hydrolyzed enzymatically with leucine aminopeptidase for 4 hours and for 24 hours; amino acid analysis of the 24-hour digestion mixture showed equimolar quantities of

\[
\begin{align*}
\text{Lys} & \quad 65 \\
\text{Val-Leu-Gly-Ala-Asp} & \quad 66 \\
\text{Gly-Leu-Ala-Ile-Leu-Asp} & \quad 71 \\
\text{Asn-Leu-Lys} & \quad 72 \\
\text{Thr} & \quad 73 \\
\text{Asp} & \quad 74 \\
\text{Asn} & \quad 75 \\
\text{Lys} & \quad 76 \\
\text{Leu} & \quad 77 \\
\text{Asp} & \quad 78 \\
\text{Asn} & \quad 79 \\
\text{Lys} & \quad 80 \\
\text{Asn} & \quad 81 \\
\text{Lys} & \quad 82 \\
\end{align*}
\]
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Fig. 6. Degradation diagram of β-tryptic IX of hemoglobin A. The designation pep, as in pep 1A, indicates a peptic peptide. *, recovered as Asp after acid hydrolysis; assumed to be Asn because of electrophoretic mobility of the peptide.

Fig. 7. Degradation diagram of β-tryptic IX of hemoglobin Cchanger. *, recovered as Asp after acid hydrolysis; assumed to be Asn because of electrophoretic mobility of the peptide.

serine, asparagine, glycine, leucine, and phenylalanine. Addition of 0.02 µmole of aspartic acid to an aliquot of the mixture prior to amino acid analysis resulted in a separate peak at the appropriate elution time, as determined from a standard amino acid mixture. Analysis of the 4-hour leucine aminopeptidase digestion mixture also showed serine, asparagine, glycine, leucine, and phenylalanine, but the yield of amino acids varied, with phenylalanine present in largest amount and leucine in smallest yield. Hydrolysis of peptides by leucine aminopeptidase proceeds from the NH2-terminal end (30). The yields suggest that at 4 hours there was incomplete digestion of the peptide, the sequence of which, from the NH2-terminal, was presumed to be that shown in Scheme 3. Thus asparagine replaced aspartic acid as the βα residue of Hb Cm. Specific stains of all peptide maps made from trypptic digestes of Hb A, Hb B2, and their β chains showed no differences in occurrence of arginine, histidine, tryptophan, and sulfur. The β1p I and β1p IX peptides from both βA and βB stained positively for histidine.

Comparison of peptide maps made from trypptic digestes of amino ethylated βB and βA chains showed no abnormalities in the position of the core peptides of βB. Peptide maps of trypptic digestes of whole globin from hemoglobins Cm and A showed no differences in the α-tryptic peptides of the two hemoglobins.

Other Studies—The sedimentation coefficient (s20, w) of hemoglobin Cm was 4.2 S as compared with 4.1 S for hemoglobin A, consistent with a tetrameric structure. Preliminary observations on the oxygen equilibria of electrophoretically isolated Hb Cm showed no significant differences from isolated Hb A in the n value (heme-heme interaction) from Hill’s equation or in the Bohr effect. A slight increase in oxygen affinity of hemoglobin Cm as compared with Hb A has been noted (e.g. p50 at 10° in 0.1 M phosphate buffer, pH 7.2, for Hb Cm = 1.8 mm Hg, for Hb A = 2.6 mm Hg): further studies are necessary to establish the significance of the differences in oxygen affinity. Hemoglobin Cm did not differ from Hb A in its visible and ultraviolet spectra in the oxy and deoxy states and in titration with HMB which showed two reactive sulfhydryl groups per tetramer (calculated values were 2.5 for Hb Cm and 2.4 for Hb A). With these techniques, no evidence of gross conformational difference between Hb Cm, Hb S, and Hb A was found.

Observations of Sickling Properties—In addition to erythrocyte sickling in routine preparations (cells in 2% sodium metabisulfite, or a film of 0.9% NaCl-suspended cells sealed under a cover slip), almost 100% sickling was observed within 20 min when 1 drop of the red cells of the propositus, suspended in phosphate buffer at pH 7.2, was deoxygenated in a chamber with nitrogen; the erythrocytes reverted to normal shape on exposure to oxygen. Most of the sickled cells had the frayed ends (holly leaf forms) (Fig. 8) commonly encountered in sickled red cells from individuals with sickle trait, but a few filamentous forms were seen. Cooling the chamber in an ice bath prevented sickling during exposure to...
FIG. 8. Sickling of the erythrocytes of the propositus. Erythrocytes were suspended in isotonic 0.9% NaCl under a sealed coverslip for 24 hours.

FIG. 9. Salting out curves of isolated hemoglobins S and C$_{Harlem}$. Solubilities were determined at 25° in phosphate buffer at pH 6.8. The ionic strength of the Na$_2$HPO$_4$ is included in the total ionic strength for the deoxygenated hemoglobins.

Nitrogen for 2 hours; subsequent warming to room temperature resulted in almost complete sickling within 10 min. Under the polarizing microscope, the sickled cells of the propositus, as those containing Hb S (31), showed a negative birefringence in the direction of the long axis of the sickle cell, independent of the membrane birefringence. The oxygenated, unsickled cells were not birefringent.

**Solubility Determination**—Comparison of the solubilities of isolated hemoglobins C$_H$ and S in the oxy and deoxy states (Fig. 9) revealed that the deoxy form of each was far less soluble than the oxy form, although deoxy-Hb C$_H$ appeared slightly more soluble than deoxy-Hb S. It is possible that Hb A$_S$, which is
present in an undetermined amount (possibly as much as 7%) in the isolated Hb CH could account for this difference.

Gelation Studies—The minimum hemoglobin concentrations required for gelation upon deoxygenation were determined for hemolysates from erythrocytes of the propositus, for persons with sickle cell anemia and sickle trait, and for prepared mixtures of the isolated hemoglobins. Comparison of the minimum gelling concentrations of mixtures of Hb S + Hb A and Hb Cn + Hb A (Table III; Fig. 10) revealed that, with mixtures containing over 50% Hb A (including hemolysates obtained from erythrocytes of patients with 40% Hb S or Cn and 60% Hb A), hemoglobins S and Cn behave the same: the greater the proportion of Hb A, the smaller the concentration of hemoglobins S or Cn required for gelation, while the total concentration increases. These data resembled those obtained for Hb S by Singer and Singer (22). However, isolated Hb Cn required a much higher concentration to gel (36.2 g/100 ml) than did Hb S (24.5 g/100 ml). Furthermore, the reduction in minimum gelling concentration of total hemoglobin when small proportions of Hb A were added to Hb Cn represented a qualitative difference between the behavior of the Hb Cn and that of Hb S. For the latter, the lowest total hemoglobin concentration for gelling occurred in the absence of other hemoglobins (22, 32).

All gels containing Hb Cn liquified upon cooling to near 0° and gelled again with warming, as occurs with Hb S.

In order to study the interaction of Hb CH and Hb S, minimum gelling concentrations were determined for mixtures of these two isolated hemoglobins. Comparison with mixtures of the same proportions of each with Hb A (Table IV; Fig. 11) showed that mixtures containing Hb CH and Hb S gelled at consistently lower concentrations than either component mixed with Hb A, but no mixture gelled at significantly lower concentration than Hb S alone.

**DISCUSSION**

The new hemoglobin variant described contains two amino acid substitutions in each β-polypeptide chain: valyl for glutamyl at β6 as in Hb S and asparaginyl for aspartyl at β73. Two substitutions in a single polypeptide chain have not been previously defined for any hemoglobin variant; they could have originated either by a second mutation in a β chain gene which already carried a single mutation at the site for β6 or β73, or by homologous crossing over within the β chain locus in an individual doubly heterozygous for Hb S (αββ′, Ψ) and for a hypothetical hemoglobin with the structure α′(Ψ)ββ′(Ψ)Am.

Each substitution in Hb CH is consistent with a one-step mutation (a single base substitution) in the triplet code of messenger RNA, as has been observed with each of the human hemoglobin variants with a single amino acid substitution (1). It may be of interest to note that indirect evidence recently presented by Gerald and Rath (33) suggests that Hb Clements, originally believed to be αββ′, Ψ, is another example of sickle hemoglobin with a second β chain substitution. The location of the second substitution was not yet established.
evolutionary interest that the position in the \(\alpha\) chain which is homologous to \(\beta^B\) at \(\alpha^6\) (34) is occupied by asparagine (17).

Like hemoglobin \(S\), hemoglobin \(C_H\) was associated with erythrocyte sickling and relative insolubility of the deoxygenated form, but significant differences were observed in the gelation properties of the two deoxyhemoglobins. Since the initial observations of Harris and Bunting (35), that concentrated solutions of hemoglobin \(S\) will undergo gelation on deoxygination, gelling techniques have been utilized for studies of hemoglobin variants (22, 32, 36) and minimum hemoglobin concentrations required for gelation have been taken as a measure of interactions between molecules of deoxyhemoglobin. The significantly higher concentration required for gelation of isolated \(Hb\ C_H\) as compared with \(Hb\ S\) suggested that the \(\beta^B\) substitution in \(Hb\ C_H\) resulted in decreased intermolecular interactions in solutions of this deoxyhemoglobin, and implied conformational differences between the deoxyhemoglobins \(C_H\) and \(S\).

In contrast to the differences observed in gelation of isolated \(Hb\ S\) and \(Hb\ C_H\), little difference in minimum gelling points was observed between mixtures of hemoglobins \(C_H\) and \(A\) and mixtures containing similar proportions of hemoglobins \(S\) and \(A\). Thus when the proportion of \(Hb\ A\) amounted to more than one half of the mixture (as would be observed in the erythrocytes of \(Hb\ \alpha\)-thalassemia trait), the minimum gelling point in the absence of \(Hb\ A\) resulted in decreased intermolecular interactions in solutions of this deoxyhemoglobin, and implied conformational differences between the deoxyhemoglobins \(C_H\) and \(S\).

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