β-SHEET AGGREGATION PROPOSED IN SICKLE CELL HEMOGLOBIN*

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Received September 18, 1974

Summary: A β-sheet conformation is predicted at the N-terminal of β chains in sickle cell hemoglobin (Hb S) as a result of the β6 Glu → Val mutation. Since Glu is the weakest and Val is the strongest β-sheet former in the predictive method of Chou and Fasman [Biochemistry 13, 211, 222 (1974)], such a substitution greatly increases the β-sheet potential in the β 1-6 region. The similarity in the concentration and temperature dependence of Hb S gelation to β-sheet formation in polyamino acids suggest that a common aggregation mechanism may be involved. Conditions to cause a β → α transformation at the β 1-6 region of Hb S is discussed relative to the treatment of sickle cell disease.

Introduction

Sickle cell hemoglobin (Hb S) differs from normal human adult hemoglobin (Hb A) in that Val is substituted for Glu at the sixth position from the N-terminal of both β chains (1). This mutation causes aggregation of Hb S inside the erythrocyte upon deoxygenation, resulting in the sickling phenomenon. An earlier proposed Hb S structure based on intramolecular cyclization from Val 1 to Val 6 via hydrophobic bonding (2) has been revised by Murayama in favor of an intertetrameric hydrophobic interaction at the Val β6 position (3). However, this revised model cannot satisfactorily explain the lack of sickling in Hb G Makassar (β6 Glu → Ala) (4) even though Ala is also hydrophobic (5). Recently several models of Hb S have been presented based on electron microscopy (6, 7) and polarized absorption (8). While these studies give information on the orientation of Hb S fiber formation, the stereochemical mechanism of aggregation remains unknown (6). Since high resolution X-ray diffraction studies of deoxy Hb S are still in progress (9, 10), it would be informative in utilizing the protein predictive model of Chou and Fasman (11, 12) to elucidate the conformation of Hb S around the mutation region.

Methods and Results

The novel part of the present analysis resides in the fact that

*Publication # 969 of the Graduate Department of Biochemistry, Brandeis University.

amino acids have conformational potentials (i.e., helical potential, P_α, and β-sheet potential, P_β) and that regions in proteins are capable of undergoing conformational change if their average helical potential, $<P_\alpha>$, and average β-potential, $<P_\beta>$ should alter upon mutation or solvent changes (11, 12). The conformational assignments of the amino acids as formers, breakers, and indifferent to helix and β-sheet formation (12) have been utilized in analyzing the conformation of the N-terminal β-chains in Hb A and Hb S, and are summarized in Table 1.

The β6 Glu → Val mutation in Hb S involves not only the substitution of a charged polar residue by an uncharged hydrophobic residue (2), or one helical forming residue [$(P_\alpha)_{Glu} = 1.53$] by another [$(P_\alpha)_{Val} = 1.14$], but more importantly, it causes a drastic change in β-sheet potential. The strongest β breaker, or alternatively the weakest β former [$(P_\beta)_{Glu} = 0.26$] is replaced by the strongest β former [$(P_\beta)_{Val} = 1.65$] in Hb S. Since Val 1, Leu 3, and Thr 4 in the β1-6 region are also β-sheet formers in addition to Val 6, the overall conformational change becomes $<P_\alpha> = 1.11 \rightarrow 1.05$ and $<P_\beta> = 0.94 \rightarrow 1.18$. Hence, a single amino acid mutation (β6 Glu → Val) could change the partial helical N-terminal β1-6 region (Val His Leu Thr Pro Val) into a β-sheet.

The result of this $\alpha \rightarrow \beta$ transformation at the first turn of the A-helix in the β chain of Hb S is shown schematically in Fig. 1. No perturbation of the A-helix is predicted for the β6 Glu → Ala substitution in Hb G Makassar since Ala ($P_\alpha = 1.45$, $P_\beta = 0.97$) is a strong helix former and a weak β former. The helical potential is greater than the β-potential in Hb G Makassar ($<P_\alpha> = 1.11 > <P_\beta> = 1.06$) as in Hb A ($<P_\alpha> = 1.11 > <P_\beta> = 0.94$) so that β formation in region β 1-6 is not predicted. However, in Hb S, β-sheet formation is favored since $<P_\beta> = 1.18 > <P_\alpha> = 1.05$. This difference may account for the aggregation behavior of Hb S which is absent in Hb A. Since mixtures of Hb S with Hb G Makassar showed an identical minimum gelling concentration (MGC) to that of mixtures of Hb S and Hb A (15), the conformation of Hb G Makassar and Hb A appears to be very similar in agreement with prediction.

Discussion

X-ray crystallographic studies showed that the β_1-β_2 hemes move 6.5 Å further apart upon deoxygenation (16). The α_1-β_2 contact region also undergoes drastic changes in the deoxy state as the two subunits turn relative to each other by 13° (17). These shifts in the β chains of deoxy Hb S could cause the proposed β-sheet at the β 1-6 regions to form intermolecular hydrogen bonds with complementary sites of adjacent Hb S molecules.

Table 1. Conformational prediction for the N-terminal region β1-6 of the β chains in Hb A and Hb S.

Protein	N-terminal sequence *	Hierarchical Analysis †	Average Conformational Potential ‡	
	1 2 3 4 5 6 7 8 9 10			
Hb A	Val His Leu Thr Pro Glu Glu Lys Ser Ala			
α-potential	{h h H i B H} H I i H	$(H_2h_2iB)_\alpha$	$\langle P_\alpha \rangle_{15} = 1.11$	$\langle P_\alpha \rangle_{29} = 1.03$
β-potential	{H i h h b B} B b b I	$(Hh_2ibB)_\beta$	$\langle P_\beta \rangle_{15} = 0.94$	$\langle P_\beta \rangle_{29} = 0.99$
	1 2 3 4 5 6 7 8 9 10			
Hb S	Val His Leu Thr Pro Val Glu Lys Ser Ala			
α-potential	{h h H i B h} H I i H	$(Hh_3iB)_\alpha$	$\langle P_\alpha \rangle_{15} = 1.05$	$\langle P_\alpha \rangle_{29} = 0.96$
β-potential	{H i h h b H} B b b I	$(H_2h_2ib)_\beta$	$\langle P_\beta \rangle_{15} = 1.18$	$\langle P_\beta \rangle_{29} = 1.21$

* Assignments in the first row under each residue refers to helical potential (α), and in the second row to β-sheet potential (β) : H (strong former), h (former), I (weak former), i (indifferent), b (breaker), B (strong breaker)(1). The tetrapeptide breaker (BbbI) at β7-10 limits β-sheet formation in Hb S to region β1-6, whose α- and β-potential assignments are shown in brackets for Hb A and Hb S. The β assignment for His has been changed from breaker (b) to indifferent (i) on the basis of 29 proteins with $P_\beta = 0.87$ (Chou and Fasman, unpublished results).

† The grouping of α- and β-formers, breakers, etc. provides a qualitative comparison showing that the α-potential is greater than the β-potential for β1-6 in Hb A, whereas the reverse is the case in Hb S.

‡ $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ are respectively the average conformational potential for the computed β1-6 region to be in the helical and β-sheet conformation. The subscripts 15 and 29 refer to the P_α and P_β values of 15 proteins (1) and 29 proteins (Chou and Fasman, unpublished results) respectively upon which the $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values are based.

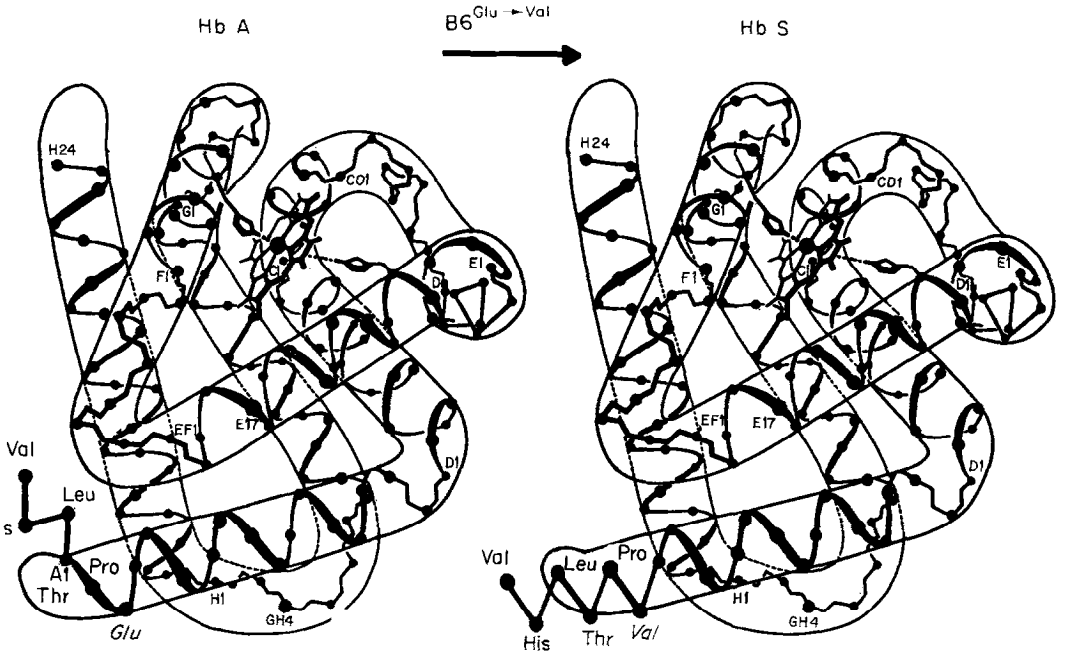

FIG. 1 Proposed $\alpha \rightarrow \beta$ transformation at the N-terminal of β chain of sickle cell hemoglobin (Hb S) when Val replaces Glu at the $\beta 6$ position of normal adult hemoglobin (Hb A). The myoglobin molecule (13) has been adapted to depict the β chain of hemoglobin which has a similar conformation (14). The $\beta 6$ Glu→ Val mutation disrupts the first turn [A1-3 (residues 4-6)] of the A helix (residues 4-18), and converts the $\beta 1$-6 region from a partial α-helix to a β-conformation. When Thr Pro Glu are in the helical form of Hb A, they are involved in intra-chain hydrogen bonding, so that inter-chain interactions are minimized. When Thr Pro Val are in the β-conformation in Hb S, they may easily participate in inter-chain β-sheet formation with adjacent Hb S molecules, since Thr 4 and Val 6 are both β-formers (12). Furthermore, the side chains of Thr 4 and Val 6 may be involved in polar and hydrophobic contacts respectively with their complementary sites.

With increasing Hb S concentration, aggregation becomes increasingly favorable until gelation occurs (MGC). β-sheet aggregation of oxy Hb S is probably prevented by the inaccessibility of β 1-6 residues to come into close hydrogen-bonding contacts between neighboring oxy Hb S molecules due to stereochemical orientation of the N-terminal β-chains.

There is some evidence from electron microscopic studies (7) that the two $\beta 6$ regions of each deoxy Hb S molecule are in contact with the $\beta 6$ regions of the molecules above and below. This would make the β-sheet interactions between the $\beta 1$-6 regions of adjacent Hb S molecules an exceedingly attractive mechanism for Hb S aggregation. However, there are gelation experiments performed with mixtures of Hb S and other mutant hemoglobins which restrict intermolecular contacts to only one $\beta 6$ Val region

while the β73 region in the other β chain is proposed as another binding site (15, 18). Although there are no β-sheets in native hemoglobin (14), there are residues in the random coil regions and at the ends of helices which may participate in intermolecular hydrogen bonding. It is possible that these residues could assume the β conformation when they interact with the β1-6 region during deoxy Hb S gelation.

Favorable and unfavorable juxtapositions of β-sheet formation in hemoglobin mutants with Hb S are proposed to potentiate and inhibit sickling respectively. Hence, the findings that Hb D Punjab (β121 Glu →Gln) and foetal hemoglobin (Hb F) interacted the most and least, respectively, with Hb S (19) may be explained by an α → β transformation in the region (β118-123) of mutation of Hb D Punjab ($<P_\beta>$= 1.09 > $<P_\alpha>$= 0.97) which could enhance β-sheet interaction. In Hb F, the helical potential is much higher than the β-potential at the γ1-6 region ($<P_\alpha>$= 1.13 > $<P_\beta>$= 0.75), so that β-sheet interaction with Hb S is diminished.

It is well known that intermolecular β-sheet aggregation in poly (L-Lys) is dependent on polymer concentration, temperature, and rate of heating (20), thus bearing striking resemblance to deoxy Hb S aggregation. Hb S solutions at 14 g/dl and higher concentrations aggregate into a gel at 20° (21). Recent calorimetric studies show that the kinetics of Hb S gelation is also dependent on temperature as well as rate of heating (22). Helical poly(L-Lys) at pH > 11 forms β-sheets at 23° - 50° depending on concentration and solvent conditions, but can revert back to the α-helical conformation at 4° (20, 23). Hence, the observation that deoxy Hb S solution at 0° gels when warmed to 38° but liquefies reversibly when replaced in the ice bath (2) may be due to a helix → β-sheet →helix transformation. Poly(L-Val)-ribonuclease also undergoes thermal aggregation at 30°-39°, whereas native ribonuclease does not, and such aggregations can be reversed in the cold (0°-4°)(24), as well as by addition of urea (25). Similarly, aggregation of Hb S (β6 Glu→ Val) can also be reversed by cooling or adding urea (3).

These aggregational phenomena may be satisfactorily explained in the light of optical rotatory dispersion (ORD) and circular dichroism (CD) studies on poly(L-Val) in aqueous solution, showing that the β-sheet is the predominant conformation (26, 27). It was found that poly(D, L-Lys)-poly (L-Val) formed large aggregates with increasing concentration, while the solubility of the polymer decreases, and a hydrogen-bonded β-sheet was suggested which aggregated by 3-dimensional stacking in H_2O (26). It was also noted that when poly(L-Val) chain becomes large in the copoly-

mer, the solution becomes too viscous and finally gels (26). Furthermore, CD studies of poly(L-Val^{23}L-Glu^{77}) showed increasing β-sheet formation upon heating (28). Potentiometric titration of poly(L-Val^{13}L-Lys^{87}) showed a coil $\rightarrow \beta$ transition at 25° (50% β at pH 9.9; 100% β at pH 10.3) with $\Delta H°_{Lys}$ = 985 cal/mole, $\Delta H°_{Val}$ = 854 cal/mole and $\Delta S°_{Lys}$ = 3.7 e.u., $\Delta S°_{Val}$ = 6.0 e.u. (Mandel and Fasman, to be published). For coil $\rightarrow$ helix transition of poly(L-Lys), $\Delta H°_{Lys}$ = -885 cal/mole and $\Delta S°_{Lys}$ = -2.7 e.u. from titration studies (29). Since Hb S aggregation was found to be endothermic (3, 22) with a positive change in entropy (3), β-sheet formation may be involved.

The β-sheet aggregation mechanism in Hb S postulated herein suggests a possible direction of therapeutic treatment in sickle cell disease. This would involve the finding of conditions which block the formation of β-sheets or cause a $\beta \rightarrow \alpha$ transformation at the β 1-6 region of Hb S, since no aggregation is observed in HbA (21). Since β-sheet formation is favored at high concentration and elevated temperatures, and α-helix favored at low temperatures, a sickle cell crisis may be alleviated by reduction of Hb S concentration and prevention of elevation in body temperature through antipyretic agents.

It has been shown that methanol can prevent poly(L-Lys) from β formation at any temperature (20) and transform β-sheets to α-helices in β-lactoglobulin (30). Similarly, the β-sheets in concanavalin A can be totally changed to α - helices by the addition of 2-chloroethanol (31). If β-sheets are involved in Hb S gelation, the use of organic solvents could cause Hb S disaggregation through a $\beta \rightarrow \alpha$ transformation.

The recent findings that ethanol inhibit the precipitation of oxy Hb S during mechanical shaking (32) as well as the gelation of deoxy Hb S and sickling of erythrocytes (32, 33) may now be better understood. Ethanol is expected to weaken β-sheet interaction through α-helix stabilization (34, 35), thus preventing Hb S aggregation in the sickling phenomenon. Although the in vitro studies showing that sickling is reduced by 50% with ethanol treatment appear promising, it should be cautioned that there have been cases where consumption of alcohol by sickle cell patients induced hemolytic crises (36, 37). Clearly more experiments along these lines, as well as toxicity and clinical studies, are necessary for a greater understanding and a possible cure of sickle cell disease.

Acknowledgments: I thank kindly Dr. G. D. Fasman for support from his grants [U. S. Public Health (GM 17533), National Science Foundation (GB

29204X), American Heart Association (71-1111) and the American Cancer Society (P-577)] ; Drs. R. Mandel and G. D. Fasman for supplying data on the Val-Lys copolymers studies, and Dr. S. J. Edelstein for bringing Hb S to my attention.

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