High-Resolution X-ray Study of Deoxy Recombinant Human Hemoglobins Synthesized from β-Globins Having Mutated Amino Termini[†,‡]

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ABSTRACT: The crystal structures of three mutant hemoglobins reconstituted from recombinant β chains and authentic human α chains have been determined in the deoxy state at 1.8-Å resolution. The primary structures of the mutant hemoglobins differ at the β-chain amino terminus. One mutant, βMet, is characterized by the addition of a methionine at the amino terminus. The other two hemoglobins are characterized by substitution of Val 1β with either a methionine, βV1M, or an alanine, βV1A. All the mutation-induced structural perturbations are small intrasubunit changes that are localized to the immediate vicinity of the β-chain amino terminus. In the βMet and βV1A mutants, the mobility of the β-chain amino terminus increases and the electron density of an associated inorganic anion is decreased. In contrast, the β-chain amino terminus of the βV1M mutant becomes less mobile, and the inorganic anion binds with increased affinity. These structural differences can be correlated with functional data for the mutant hemoglobins [Doyle, M. L., Lew, G., DeYoung, A., Kwiatkowski, L., Noble, R. W., & Ackers, G. K. (1992) *Biochemistry* preceding paper is this issue] as well as with the properties of ruminant hemoglobins and a mechanism [Perutz, M., & Imai, K. (1980) *J. Mol. Biol.* 136, 183–191] that relates the intrasubunit interactions of the β-chain amino terminus to changes in oxygen affinity. Since the structures of the mutant deoxyhemoglobins show only subtle differences from the structure of deoxyhemoglobin A, it is concluded that any of the three hemoglobins could probably function as a surrogate for hemoglobin A. However, because the structure of the βV1M mutant is perturbed the least, it is the most likely candidate for a hemoglobin A proxy.

Hemoglobin has been studied extensively as the prototypic allosteric protein with the goal of elucidating structural mechanisms by which allosteric regulation is achieved (Perutz, 1989). Future progress will depend to a large degree on our ability to test specific hypotheses with site-directed hemoglobin mutants. By correlating detailed structural information, in the form of high-resolution X-ray crystallographic structures, with accurate thermodynamic and kinetic data, it should be possible to determine how specific residues contribute to the allosteric properties of hemoglobin.

Our collaborators, Hernan et al. (1992), have developed an efficient protocol for producing large amounts of β-globin in *Escherichia coli*. However, due to amino terminal processing differences in mammals and bacteria, this protocol results in globin chains with altered amino termini. Therefore, three mutant hemoglobins have been synthesized in the hope that at least one would mimic the structure and the functional properties of Hb A[1] sufficiently well to serve as a surrogate from which further mutations can be engineered. The mutant βMet is characterized by an additional methionine at the amino terminus and βV1A by a valine to alanine substitution, and Val 1β is replaced by a methionine in βV1M. The expectation was that none of these mutations would alter greatly the functional properties of hemoglobin because the β-chain amino terminus is located on the surface and does not contribute to strong intersubunit contacts (Fermi et al., 1984). Functional characterization of the mutant hemoglobins in the second paper of this series (Doyle et al., 1992) confirmed this expectation. All three of the mutants were found to be highly cooperative and very similar to Hb A in terms of their thermodynamic and kinetic properties. There are, however, small differences in the functional properties of each of the mutant hemoglobins compared to those of Hb A. All three mutant hemoglobins have slightly lower oxygen affinities and exhibit small changes in the thermodynamics of dimer–tetramer assembly, but the βV1M mutant has the lowest oxygen affinity and the most stable deoxy tetramer.

In this paper we report the crystal structures of these mutant hemoglobins in the deoxy form at a resolution of 1.8 Å. Our findings support those of Doyle et al. (1992) in that none of the mutant structures are greatly perturbed relative to the structure of deoxyHb A. Nevertheless, small changes in structure at the β-chain amino terminus are observed, and they can be rationalized with the observed differences in functional properties.

MATERIALS AND METHODS

Crystallization of Deoxyhemoglobins and Collection of Diffraction Data. The synthesis and purification of the three recombinant mutant hemoglobins is described in the first article of this series (Hernan et al., 1992), and the procedure by which CO was photodissociated is detailed in the second article (Doyle et al., 1992). Prior to crystallization, the hemoglobins were concentrated and dialyzed into 10 mM ammonium phosphate buffer (pH 7.0). The three mutant hemoglobins were crystallized under deoxy conditions from

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[‡] Crystallographic coordinates have been submitted to the Brookhaven Protein Data Bank.

[1] Abbreviations: Hb A, human hemoglobin A; βMet, β1 + Met mutant; βV1M, β1 Val → Met mutant; βV1A, β1 Val → Ala mutant; $F_o - F_c$, $F_{observed} - F_{calculated}$; DPG, 2,3-diphosphoglyceric acid; IHP, inositol hexaphosphate; Tris, tris(hydroxymethyl)aminomethane; Na_2EDTA, disodium ethylenediaminetetraacetate; rms, root mean square.

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Table I: Unit Cell Constants, Space Group $P2_1$[a]

hemoglobin	a (Å)	b (Å)	c (Å)	β (deg)
deoxyhemoglobin A	63.3	83.6	53.8	99.4
deoxyhemoglobin β + Met	63.2	83.6	53.8	99.3
deoxyhemoglobin β1 Val → Met	63.2	83.7	53.8	99.4
deoxyhemoglobin β1 Val → Ala	63.2	83.5	53.8	99.4

[a] The uncertainty in these measurements is about 0.1 Å and 0.1°.

solutions of concentrated ammonium sulfate according to the procedure of Perutz (1968). As shown in Table I, these crystals are isomorphous with crystals of deoxyHb A.

The crystals were mounted in quartz capillaries and diffraction data were collected on a Rigaku AFC6 diffractometer fitted with a San Diego Multiwire Systems area detector. A single crystal of each hemoglobin mutant was used for data collection, and degradation due to radiation damage never exceeded 10%. The diffraction data were scaled and merged according to the method described by Howard et al. (1985). A total of 148 393 measurements were made of 46 131 independent reflections for βMet and 179 367 measurements of 48 524 independent reflections for βV1M. The βMet data are 90% complete and the βV1M data are 95% complete out to a resolution of 1.8 Å. In the case of βV1A, Friedel pairs were collected (a total of 341 064 measurements) and merged to 48 575 independent reflections, constituting 85% of the data out to a resolution of 1.75 Å and 90% of the data out to a resolution of 1.9 Å. All of the diffraction data are of good quality as judged by R_{symm}[2] values of 3.4%, 3.6%, and 3.7% on intensity for βMet, βV1M, and βV1A, respectively.

Refinement of the Mutant Structures. The refinements of the structures for the three mutant deoxyhemoglobins were carried out with the restrained least-squares program PROLSQ of Hendrickson and Konnert (Hendrickson, 1985) running on a VAX 6410. The initial model for all refinements was constructed from a refined structure of deoxyHb A. Specifically, our structure of deoxyHb A was obtained by refining the published deoxyHb A atomic model (Fermi et al., 1984) against a new 1.5-Å diffraction data set to a R[3] value of 16.8% (unpublished results). There are no significant differences between this deoxyHb A structure and the published structure of Fermi et al. (1984). Before beginning the refinements, the first 10 residues of the β chain and solvent near the β-chain amino terminus (including a sulfate molecule) were omitted from the atomic model.

Following initial rounds of least-squares refinement, consisting of eight cycles for βMet, nine cycles for βV1M, and five cycles for βV1A, the standard crystallographic R values for each of the models dropped from ~24% to ~21% for diffraction data between 6.0- and 1.7-Å resolution with magnitudes greater than 4σ. The resulting models were used to calculate $F_o - F_c$ difference Fourier maps on a VAXstation 3200, and the amino terminal residues of the β chains were fit into these images with the interactive computer graphics program TOM/FRODO (Cambillau, 1989), a version of the program FRODO (Jones, 1985) running on a Silicon Graphics workstation. Difference electron density maps calculated with the known deoxyHb A phases and $|F_{Hb\ mutant} - F_{HbA}|$ magnitudes were also used as an aid in reconstructing the β-chain amino terminus.

The remainder of the refinements consisted of iterations of least-squares refinement and manual rebuilding of the models in the vicinity of the β-chain amino terminus. Individual atomic temperature factors were refined throughout the refinement process.

Completion of the βMet refinement entailed an additional 30 PROLSQ refinement cycles and two interactive computer graphics sessions. The final deoxy-βMet model has a standard crystallographic R value of 16.0% for diffraction data with magnitudes greater than 2σ between 6.0- and 1.7-Å resolution. Two sulfate molecules (see below) were not reincorporated into the βMet atomic model because the electron density at these sites had decreased to background level. The remainder of the βV1A refinement consisted of 4 manual rebuilding sessions and 29 PROLSQ cycles, and the two sulfate molecules were included in the model for the last 10 least-squares refinement cycles. The final βV1A model has a standard crystallographic R value of 15.6% for diffraction data between 6.0- and 1.7-Å resolution and magnitudes greater than 2σ. Six manual rebuilding sessions and 46 PROLSQ cycles, with two sulfate molecules included in the model for the last 21 PROLSQ cycles, completed the βV1M refinement. The standard crystallographic R value for the final βV1M model is 16.0% for data between 6.0- and 1.7-Å resolution and magnitudes greater than 2σ. Water molecules with temperature factors greater than 50 Å^2 were deleted from the final models. All three of the models have good stereochemistry, as indicated by the refinement statistics in Table II.

Table II: Summary of Least-Squares Refinement Parameters (Hendrickson, 1985)

parameter	target σ[b]	rms Δ[a] Hb A	β Met	βV1M	βV1A
bonding distance (Å)					
bond length (1–2 neighbors)[c]	0.010	0.014	0.013	0.011	0.011
1–3 neighbor distances[d]	0.015	0.030	0.029	0.025	0.025
1–4 planar neighbor distances[e]	0.030	0.053	0.048	0.042	0.043
planar groups					
deviations from plane (Å)	0.010	0.014	0.013	0.011	0.012
chiral centers					
chiral volumes (Å^3)	0.080	0.151	0.164	0.144	0.144
nonbonded contacts (Å)					
separated by one torsion angle	0.200	0.166	0.167	0.162	0.163
other van der Waals contacts	0.200	0.168	0.183	0.171	0.171
possible hydrogen bonds	0.200	0.137	0.158	0.151	0.155
conformational torsion angles (deg)					
planar (e.g., peptide ω)	5.0	3.1	3.9	3.6	3.1
staggered (e.g., aliphatic χ)	15.0	19.5	21.1	21.8	21.4
transverse (e.g., aromatic χ_2)	25.0	32.3	33.6	32.5	33.2
isotropic temperatures (Å^2)					
main-chain (1–2 neighbors)[c]	2.0	2.4	2.7	2.3	2.5
main-chain (1–3 neighbors)[d]	3.0	3.4	3.7	3.2	3.5
side-chain (1–2 neighbors)[c]	4.0	7.5	7.4	6.8	6.9
side-chain (1–3 neighbors)[d]	6.0	10.7	10.3	9.7	9.9

[a] rms Δ, root-mean-square deviation from ideal values as determined from accurate small-molecule crystal structures in the case of bonding distances, chiral volumes, and nonbonded contacts, or from average values in the case of isotropic temperature factors. [b] Target σ, estimated standard deviations, where $1/\sigma^2$ is used as a relative weighting factor in the minimized sum of observational functions. [c] 1–2 neighbors, covalently bonded atom pairs. [d] 1–3 neighbors, atom pairs separated by two covalent bonds. [e] 1–4 planar neighbors, atom pairs in a planar group separated by three covalent bonds.

RESULTS

The results of our X-ray crystallographic analysis indicate that while the nature of the structural perturbations differs

[2] $R_{symm} = \Sigma_{hkl}[|\overline{I_{hkl}} - I_{hkl}|]\Sigma_{hkl}\overline{I_{hkl}}$.
[3] R value $= \Sigma||F_o| - F_c||/\Sigma|F_o|$.

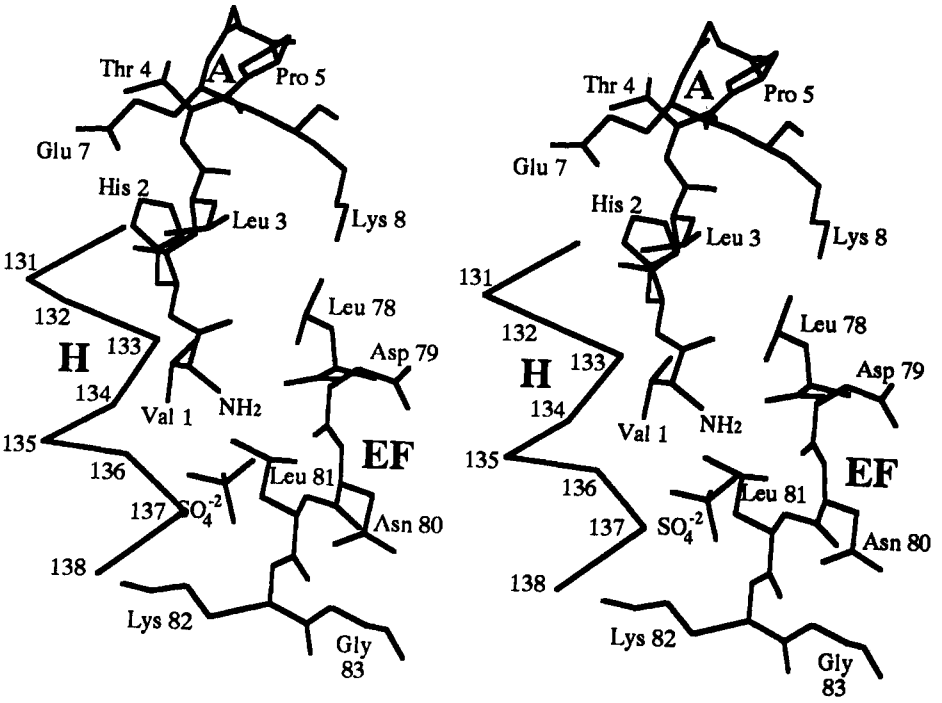

FIGURE 1: Stereo diagram of the amino terminal environment in deoxyHb A. Pictured are amino terminal residues 1–8, EF corner residues 78–83, and an C_α tracing of H helix residues 130–138. A bound sulfate anion is also shown. The side chain of Glu 6β has been omitted for clarity.

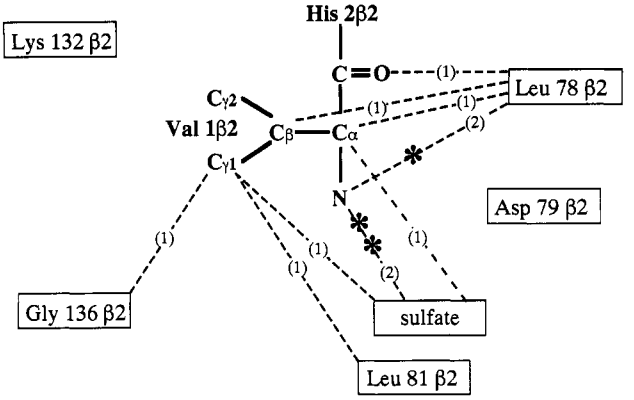

FIGURE 2: Schematic representation of atomic interactions of 4 Å or less made by Val 1β in deoxyHb A. The interactions are indicated by the dashed lines, and the numbers in parentheses denote the number of atoms that interact with a particular atom of the amino terminus. Interactions involving charged or polar atoms are indicated by an asterisk.

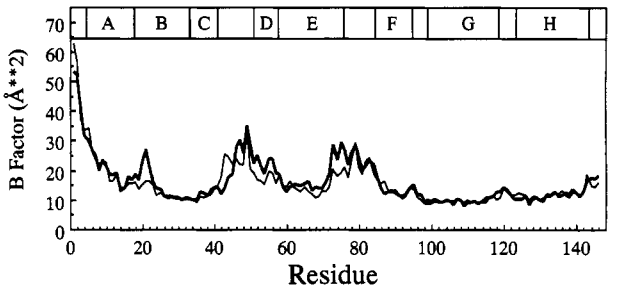

FIGURE 3: Average main-chain temperature factors plotted versus residue number for the β1 chain (thin line) and β2 chain (thick line) of deoxyHb A.

slightly for each mutant hemoglobin, in all cases the perturbations are small and they are restricted to the immediate vicinity of the β-chain amino terminus. The structural perturbations involve modifications in both the positions and the mobilities of the amino terminal residues of the β-chains. In addition, changes can be detected in the relative affinity of each hemoglobin for an inorganic anion that is normally bound to the amino terminal group in deoxy Hb A.

β-Chain Amino Termini in DeoxyHb A. In deoxyHb A (Fermi et al., 1984), Val 1β is at the entrance to the central cavity and is part of the DPG-binding site (Arnone, 1972). More specifically, the side chain of Val 1β occupies a hydrophobic pocket composed of residues from the EF corner and the H helix of the same β chain (Figure 1). The α-amino group also interacts with the EF corner by forming a hydrogen bond with the carbonyl oxygen of Leu 78β. Figure 2 schematically depicts the atomic interactions of 4.0 Å or less that are made by Val 1β. In addition to forming contacts with the EF corner and H helix, Val 1β interacts with an inorganic anion that binds between Val 1β and residue Lys 82β (Figures 1 and 2). The composition of the crystallization solution requires that this anion be either sulfate or phosphate. (The deoxyHb A model used in this study includes a sulfate anion at this site.) The electron density at the anion site is greater in the β2 chain, where the average temperature factor for the sulfate is ~60 Å^2, than in the β1 chain, where the average sulfate temperature factor has doubled to ~120 Å^2. This difference in temperature factors is in agreement with the higher temperature factors for residues at the β1 amino terminus relative to residues at the β2 amino terminus of deoxyHb A, although these later differences are smaller (Figure 3).

Deoxy-βMet versus DeoxyHb A. The structural perturbation in βMet involves a shift of the β-chain amino terminus away from the hydrophobic pocket between the EF corner and the H helix (Figure 4). This results in increased mobility of the β-chain amino terminal residues (Figure 5A). While the sulfur and C_ϵ of the methionine on the β subunit make several nonpolar interactions with residues of the H helix, the other methionine atoms extend away from the EF corner and H helix toward the central cavity. The main-chain atoms of Val 1β also move away from the EF corner and H helix, breaking all but a couple of the interactions that existed between the Val and these residues in deoxyHb A (Figure 2).

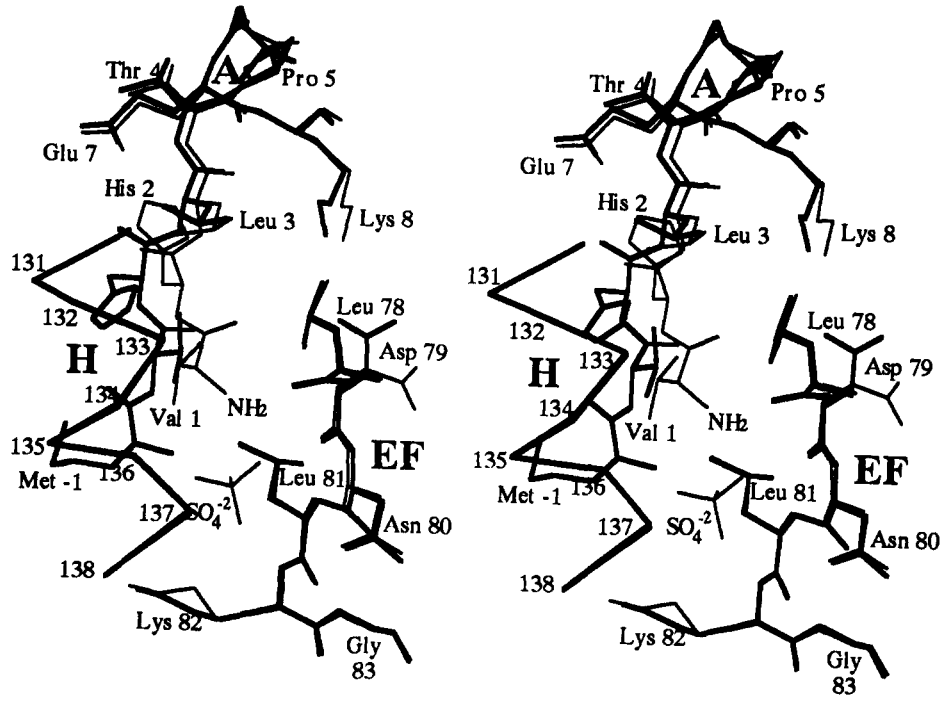

FIGURE 4: Stereo diagram of the deoxy-βMet model (thick bonds) overlaid on the deoxyHb A model (thin bonds). The view is the same as in Figure 1. The additional methionine in βMet is designated Met-1.

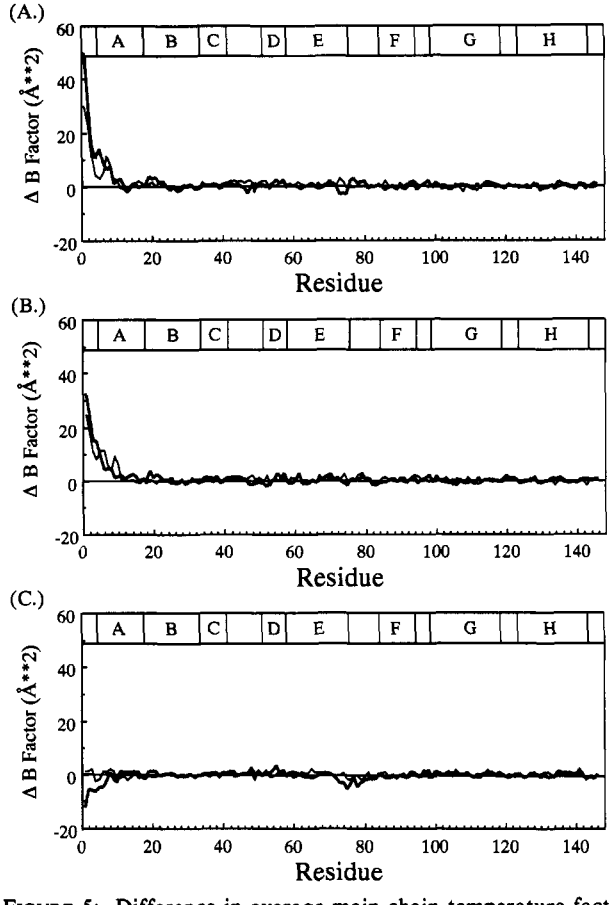

FIGURE 5: Difference in average main-chain temperature factors (mutant temperature factor minus Hb A temperature factor) plotted versus residue number for the β1 chains (thin lines) and β2 chains (thick lines) of βMet (A), βV1A (B), and βV1M (C).

These shifts extend to the beginning of the A helix before tapering off. The increased mobility of the amino terminus in deoxy-βMet correlates with reduced electron density at the anion-binding site associated with the amino terminus (Figure 6). The lack of any substantial electron density in a deoxy-βMet Fourier map (data not shown) implies that extension of the β-chain amino terminus by one methionine residue essentially eliminates the anion-binding site.

Deoxy-βV1A versus DeoxyHb A. The deoxy-βV1A structure shares some common features with the deoxy-βMet structure, namely, increased mobility of the β-chain amino terminus (Figure 5B) and reduced affinity for anions (Figure 7). In deoxy-βV1A, as in deoxy-βMet, the average main-chain temperature factors for the β-chain amino terminal residues have increased relative to those in deoxyHb A, but the magnitude of the increase is somewhat smaller in βV1A than in βMet (Figure 5B). The refined temperature factors for the sulfates are a measure of the reduced electron density; the average temperature factor for the sulfate bound to the β2 chain increases from 60 to 107 Å^2.

In deoxy-βV1A the main-chain atoms of Ala 1β move toward, rather than away from, the EF corner, so that the α-amino group interacts with the carboxyl group of Asp 79β. This results in a large reorientation of His 2β, so that it points toward the bound sulfate, as well as a decrease in mobility for the side chain of Asp 79β (Figure 8). Therefore, the reduced anion binding in βV1A appears to be due to a competition between the sulfate and the carboxyl of Asp 79β for the positive charge of the amino terminus. In deoxy-βV1A, Asp 79β appears to have won this competition, and the α-amino group moves away from the anion-binding site. This implies that in deoxyHb A the side chain of Val 1β functions as a anchor for the β-chain amino terminus, restricting its movement and therebyfavoring the binding of anion. This mechanism is different from the mechanism at work in deoxy-βMet, where the α-amino group of Met 1β has moved toward the central cavity, breaking any interactions between the EF corner residues and the amino terminus.

Deoxy-βV1M versus DeoxyHb A. In contrast to deoxy-βMet and deoxy-βV1A, where the β-chain amino terminus becomes more mobile and the affinity for anions decreases, deoxy-βV1M is characterized by a small decrease in the

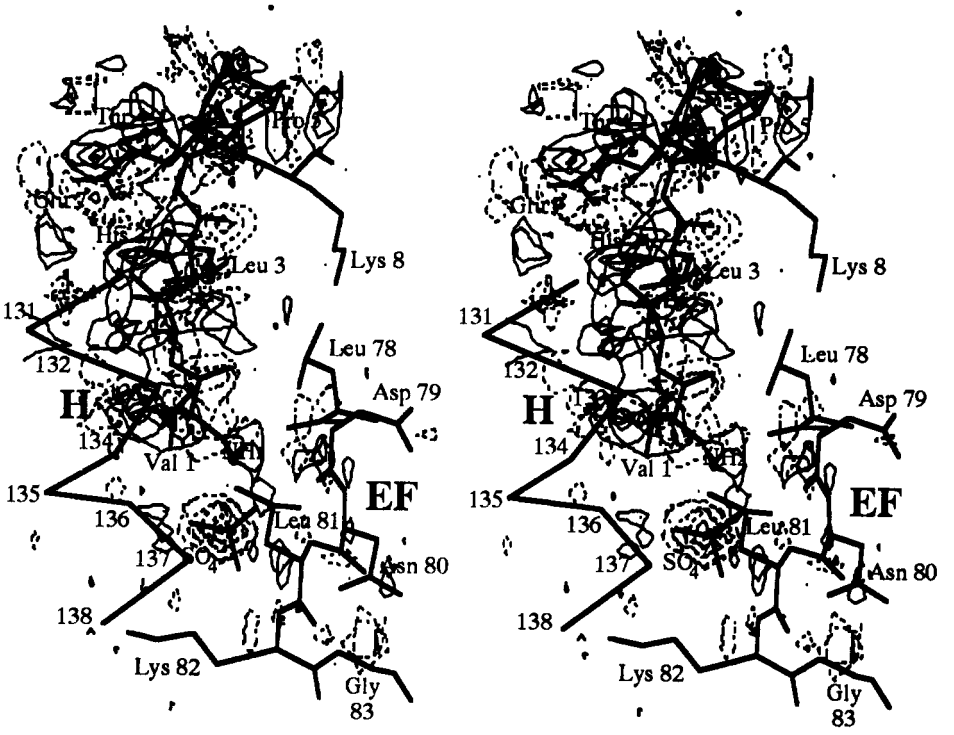

FIGURE 6: βMet – Hb A difference electron density map superimposed on the deoxyHb A atomic model. The difference electron density map was calculated with deoxyHb A phases and $|F_{\beta Met} - F_{HbA}|$ magnitudes. Positive (solid contours) and negative (dashed contours) difference electron density contours are drawn in increments of 2 times the rms value of the difference map. The large negative electron density peak over the sulfate implies a decrease in binding affinity. The absence of a strong, positive difference electron density peak for the amino terminal methionine is due to the high mobility of this residue.

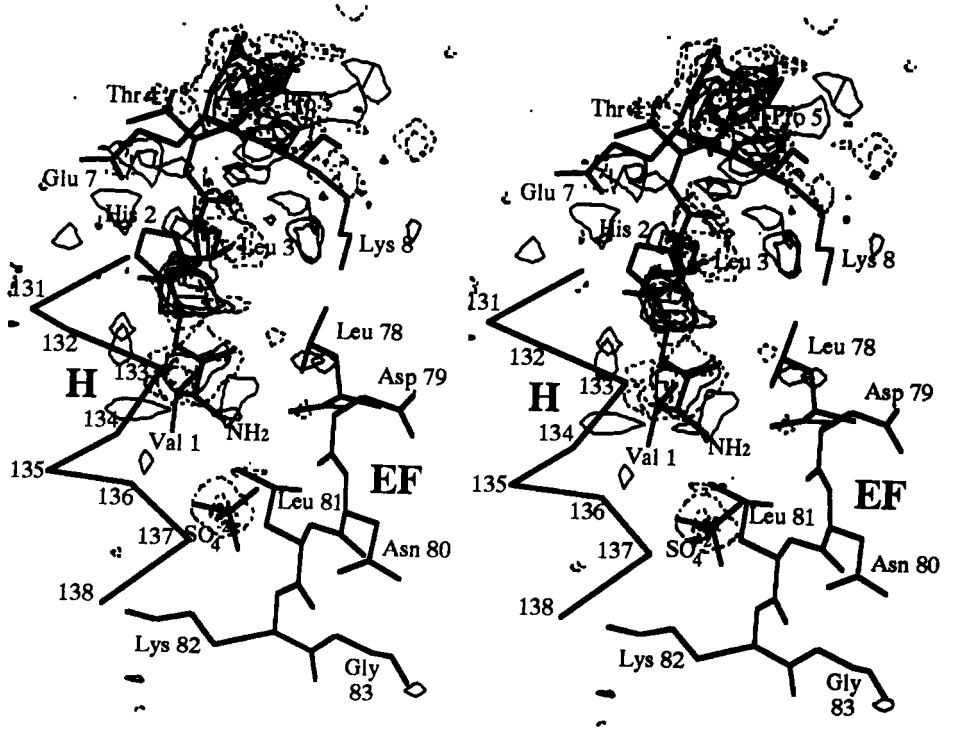

FIGURE 7: βV1A – Hb A difference electron density map superimposed on the deoxyHb A atomic model. The difference electron density map was calculated with deoxyHb A phases and $|F_{\beta V1A} - F_{HbA}|$ magnitudes. Contours are drawn as in Figure 6. Negative difference electron density contours over the sulfate are indicative of decreased binding affinity.

mobility of the β-chain amino terminus and increased affinity for inorganic anions. The deoxy-βV1M – deoxyHb A difference map (Figure 9) clearly shows added electron density for the methionine side chain and increased electron density for the bound sulfate. A plot of the change in average main-chain temperature factors for the β-chains of deoxy βV1M (Figure 5C) shows that the mobility of the β1-amino terminus is comparable to that of the β1-amino terminus in deoxyHb A and that the mobility of the β2-amino terminus has decreased slightly relative to that of the β2-amino terminus in deoxyHb A. In particular, the average main-chain temperature factor for Met 1β2 is 41 Å^2 in βV1M compared to 53 Å^2 for Val 1B2 in Hb A. This decrease in mobility correlates with increased electron density at the anion-binding site. The average tem-

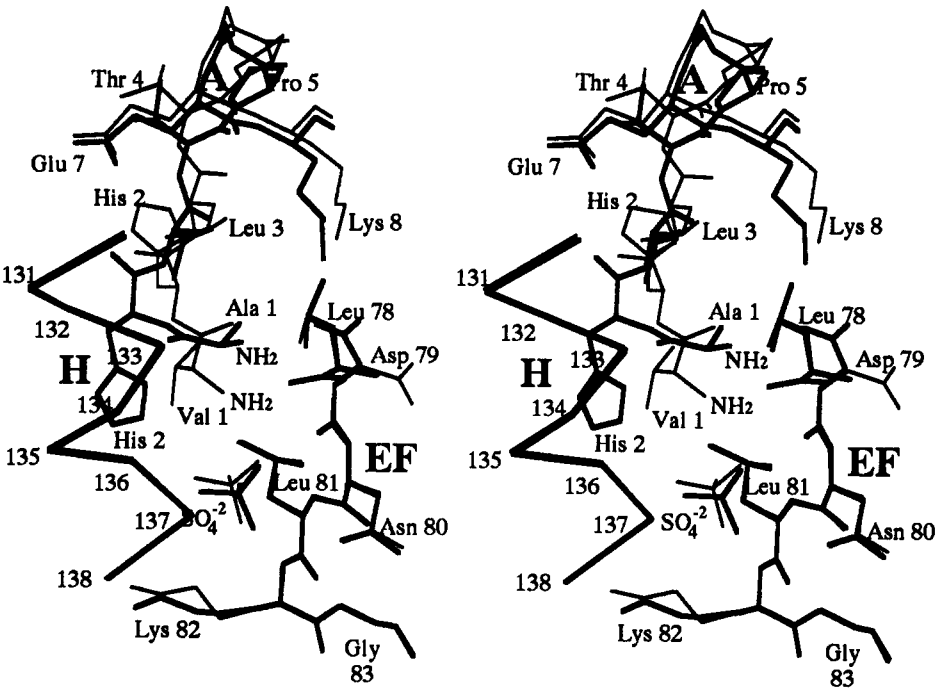

FIGURE 8: Stereo diagram of the deoxy-βV1A model (thick bonds) overlaid on the deoxyHb A model (thin bonds). The view is the same as in Figure 1.

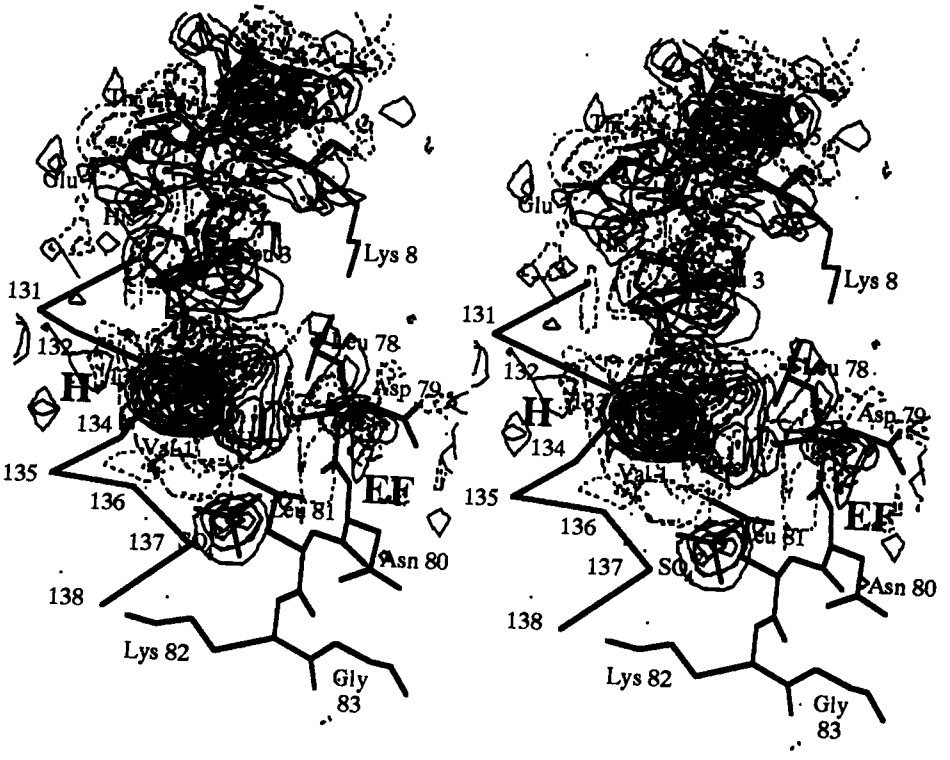

FIGURE 9: βV1M – Hb A difference electron density map superimposed on the deoxyHb A atomic model. The difference electron density map was calculated with deoxyHb A phases and $|F_{\beta V1M} - F_{HbA}|$ magnitudes. Contours are drawn as in Figure 6. The large positive electron density peak for the side chain of Met 1β indicates the position of the S atom. Positive electron density contours extending toward the EF corner show the positions of the Met 1β main-chain atoms. An increase in electron density for the bound sulfate is indicated by positive electron density contours.

perature factor for the sulfate bound to the β1 chain has decreased from 120 to 100 Å^2, and the average temperature factor for the sulfate bound to the β2 chain has decreased from 60 to 47 Å^2 as a result of the valine to methionine substitution.

In Figure 10 it is apparent that there is a small shift in the main-chain atomic coordinates as a result of the valine to methionine substitution. This perturbation is smaller in magnitude than the shifts caused by either the valine to alanine substitution or the addition of an extra methionine. The main chain has shifted away from the H helix in order to accommodate the methionine side chain, which forms several new contacts with residues on the H helix. The methionine main-chain atoms also make additional contacts with residues on the EF corner. In particular, a new interaction is formed between the α-amino group and the carbonyl oxygen of Asp

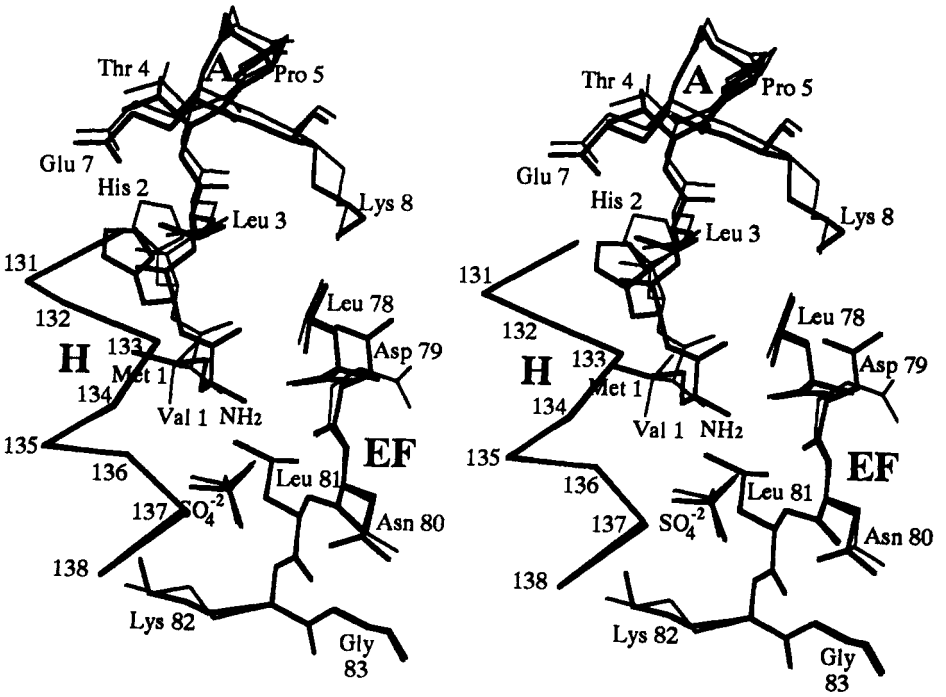

FIGURE 10: Stereo diagram of the deoxy-βV1M model (thick bonds) overlaid on the deoxyHb A model (thin bonds). The view is the same as in Figure 1.

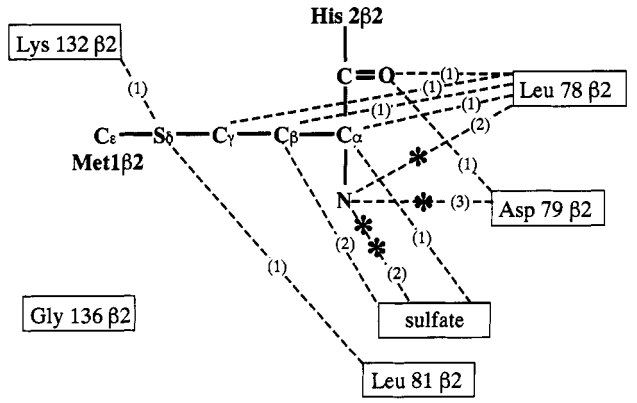

FIGURE 11: Atomic interactions of 4 Å or less made by Met 1β in deoxy-βV1M. Interactions are shown as dashed lines where the numbers in parentheses indicate the number of atoms contributing to an interaction. An asterisk denotes an interaction between polar or charged atoms.

79β. This interaction, as well as the other interactions made by the methionine are depicted schematically in Figure 11.

DISCUSSION

Suitability as a Surrogate System for Hb A. The three β-chain amino terminal mutations described above—βMet, βV1A, and βV1M—cause only small, localized perturbations to the structure of deoxyHb A. Moreover, the most critical regions of the hemoglobin tetramer, the $\alpha^1\beta^2$ interface (Pettigrew et al., 1982; Turner et al., 1992) and the heme pockets, are completely unperturbed. The overall structural identity of these mutant hemoglobins with deoxyHb A is consistent with the relatively normal functional properties reported in the previous paper (Doyle et al., 1992). Therefore, any one of the mutant hemoglobins should function quite well as a surrogate for Hb A. However, the deoxy-βV1M structure is the least perturbed of the three mutants. This together with the fact that βV1M has a slightly higher level of cooperativity probably makes it the best choice for a Hb A proxy.

Mechanistic Implications of the Structural Perturbations at the β-Chain Amino Terminus. Although the β-chain amino terminal mutations studied in this paper do not result in large changes to the structural or functional properties of hemoglobin, the energetically significant differences that have been detected may provide some insight into the way in which subtle structural perturbations are coupled to the oxygen affinity and stability of the hemoglobin tetramer.

The βMet and βV1A mutations result in hemoglobins with essentially normal oxygen affinity, cooperativity, and deoxy-subunit assembly free energies (Doyle et al., 1992). In contrast, the βV1M mutation is characterized by reduced oxygen affinity [the overall tetramer oxygenation free energy for βV1M differs from that of Hb A by +1.2 kcal (Doyle et al., 1992)] and a more stable deoxy tetramer [the deoxy dimer-to-tetramer assembly free energy for βV1M differs from that of Hb A by –0.85 kcal (Doyle et al., 1992)]. In the past, it has been argued that mutations that reduce the net positive charge at the DPG-binding site should lead to increased stability of the T quaternary structure and, therefore, reduced oxygen affinity (Bonaventura et al., 1974a,b, 1976; Moo-Penn et al., 1977). This explanation requires that the net reduction in positive charge stabilize the T quaternary structure to a greater extent than the R quaternary structure. However, this may not be true in general because the DPG-binding site narrows as a result of the T-to-R quaternary transition (Baldwin & Chothia, 1979; Shaanan et al., 1983; Fermi et al., 1984). Moreover, this theory does not explain the decreased oxygen affinity and increased stability of the deoxy tetramer in the βV1M mutant.

An alternative mechanism has been proposed by Perutz and Imai (1980) that relates the intrasubunit interactions of the β-chain amino terminus to changes in oxygen affinity and changes in the stability of the T-state quaternary structure. They proposed that the reduced intrinsic oxygen affinity of ruminant hemoglobins (compared to other mammalian hemoglobins) arises as a result of having a large hydrophobic residue, Leu, Met, or Phe, at position NA2 of the β chains; for example, the β chain of bovine hemoglobin has an amino

terminal sequence of Met2-Leu3 instead of the sequence Val1-His2-Leu3 found in human β chains. According to their hypothesis, a large hydrophobic side chain at position NA2 results in a shift of the amino terminus and the A helix closer to the EF corner and H helix, leading to tighter packing in this region, and mimicking the effect of DPG (Arnone, 1972). This tighter packing stabilizes the β-chain tertiary structure, which in turn preferentially stabilizes the T-state quaternary structure, resulting in the increased stability of the deoxy tetramer and decreased oxygen affinity that are characteristic of ruminant hemoglobins (Fronticelli, C. et al., 1988).

The structural results obtained for the deoxy-βV1M mutant hemoglobin are consistent with such a mechanism. We found that the βV1M mutation creates new intrasubunit interactions that decrease the mobility of the amino terminal peptide and the beginning of the A helix relative to the mobility of the same regions in deoxy-βMet, deoxy-βV1A, and, to a smaller extent, deoxyHb A. Moreover, the increased stability of the amino terminal peptide in deoxy-βV1M is also reflected in the increased electron density of an associated inorganic anion. In this connection, it is interesting to note that Fronticelli et al. (1988) found that under physiological conditions the low oxygen affinity of bovine hemoglobin is the result of increased oxygen-linked binding of chloride anions.

The question that remains to be answered is how the observed changes in tertiary structure are linked to the stabilization of quaternary structure. A recent normal-mode analysis of deoxymyoglobin (Seno & Gō, 1990) may provide some insight into this question. Seno and Gō identified 55 residues in the globin fold that have strong dynamic interactions with the heme. In particular, they found that clusters of residues on the A and H helices, residues that are affected by the βV1M mutation, have strong dynamic interactions with the heme despite the fact that they are 6 Å or more away from the heme. In addition, since many of the residues in the group of 55 are also part of the $\alpha^1\beta^2$ interface in hemoglobin, their analysis provides a mechanism for coupling conformational changes at the amino terminus of a globin subunit with changes in the stability of the $\alpha^1\beta^2$ interface.

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Registry No. HbA, 9034-51-9; Met, 63-68-3; Ala, 56-41-7; Val, 72-18-4; sulfate, 14808-79-8.