Hemoglobin St. Louis (β28(B10)Leu → Gln)

CRYSTAL STRUCTURE OF THE FULLY REDUCED (DEOXY) FORM

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ABSTRACT The three-dimensional structure of fully reduced Hb St. Louis has been determined to 3.5 Å resolution. The difference electron density map clearly shows the site of the mutation and the effects it produces. Glutamine B10 and histidine E7 (the distal histidine) swing towards each other and, between them, stabilize a water molecule in the normally hydrophobic heme pocket. This creation of an aqueous microenvironment near the heme accounts for the thermal instability, high rate of methemoglobin formation, and increased oxygen affinity observed in solution studies of the mutant as described in the preceeding paper. Other than a small increase in tilt of the heme, virtually no further stereochemical disturbances result.

INTRODUCTION

In recent years, knowledge of the three-dimensional structure of the hemoglobin molecule has had a profound effect on our understanding of its functional properties in vivo (1). On the basis of atomic models, Perutz and Lehmann (2) demonstrated that the majority of abnormal human hemoglobins produce physiological disorders of a kind and magnitude (usually nil) in line with the structural significance of the mutated residues and their replacements. However, some mutants have proved too complex to interpret with certainty in this way, and in these cases it is useful to investigate the structural alteration directly by X-ray crystallography (3, 4). This method is simple and straightforward when it can be applied to a structure analogous to one already solved (i.e. normal human deoxyhemoglobin), but not when a solved analogue does not yet exist (i.e. human oxyhemoglobin). The result appears in the form of a three-dimensional contour map (Figs. 1 and 2), presenting the difference between the electron density of the variant and the normal molecule in a series of sections, like serial sections through a tissue, but on a 10,000 times smaller scale. Here I describe the structural disturbances caused by the mutation β28(B10) Leu → Gln in Hb St. Louis (5) with a view to explaining the observed high rate of methemoglobin formation and tendency towards denaturation. (6).

METHODS

Crystallization. Pure Hb St. Louis (the gift of Dr. J. Rosa) was crystallized by Dr. M. F. Perutz in the usual way (7). Large crystals were obtained, isomorphous with those of normal deoxyhemoglobin A.

Data collection and processing. A full set of 3.5-Å resolution data, including Friedel pairs, was collected on a computer-controlled Hilger & Watts diffractometer (Hilger & Watts, Inc., Morton Grove, Ill.) Two crystals were used, and their data sets scaled together with an average SD of 2.0%. When matched to the native (Hb A) intensities of Arnone and Ten Eyck,[1] Hb St. Louis exhibited a mean isomorphous difference of 4.6%. The difference electron density map was synthesized and then symmetry was averaged about the molecular twofold axis to reduce noise. The map was plotted and finally enlarged photographically onto clear plastic sheets for interleaving with the native deoxy Hb A Fourier map.

RESULTS

The mutation. The difference electron density map confirms the assignment of position β28(B10) as the site of the mutation. The residue which replaces leucine at this point is seen to be larger than leucine (extending into positive peak *P* of Fig. 1) and appears to be involved in a hydrogen-bond system (notice the movement towards it of His E7(63)β in Fig. 2). These indications favor replacement of leucine by glutamic acid or glutamine, in agreement with the finding of glutamine by sequencing methods (5).

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[1] Arnone, A., and L. F. Ten Eyck. 1976. In preparation.

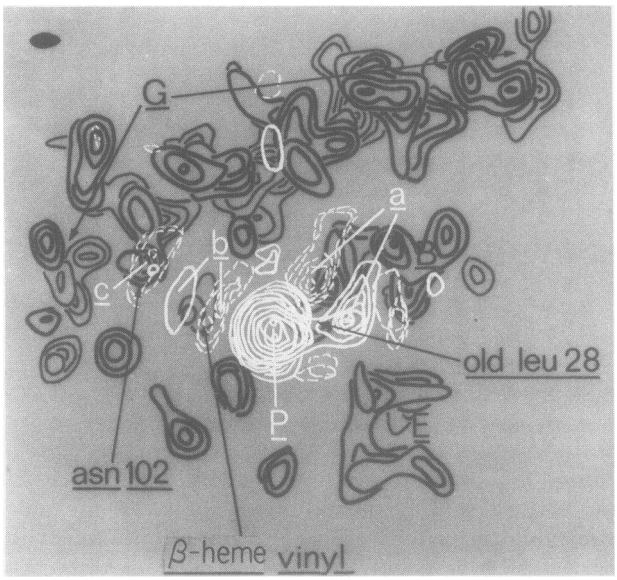

FIGURE 1 Sections $y = 0$ to -4 of the deoxyHb St. Louis difference map (white contours, 0.04 electrons/Å^3) superimposed on the deoxyHb A density map (black contours, 0.4 electrons/Å^3). Solid white contours indicate added electron density, while dotted white contours show density taken away in the mutant. Capital black letters label the G, B, and E helices of the β chain; the black crystallographic symbol at upper left marks the molecular twofold symmetry axis, which extends up, perpendicular to the page. The nearby α chain is above and to the left of this figure, with the other α and β related by a 180° rotation about the two fold. Features labeled with white letters are explained in the text.

Disturbances produced in the heme pocket. The stereochemistry of disturbances brought about by this replacement is quite simple. Glutamine side chains form strong hydrogen bonds when the opportunity exists, yet in the heme pocket, this one is practically surrounded by nonpolar hydrophobic groups. Its only hydrogen-bonding neighbor is the distal histidine (63β), which swings inwards (*b* in Fig. 2) in an attempt to make contact. However, the two groups are too far apart to form a good bond without introducing large distortions in the α-helices to which they are attached. The problem is solved by the introduction of a water molecule, hydrogen-bonded between Gln 28β and His 63β (shown schematically in Fig. 3). In this way two bonds result instead of one, producing a relatively more stable configuration. The water molecule has in a sense joined the structure of the protein.

There is a very limited amount of space in the heme pocket since it is designed (in the normal molecule) to keep water away from the heme, though allowing access by dissolved gas molecules. The position of the water molecule peculiar to this mutant is not at the oxygen binding site (at the centre of the heme) but rather above it at a point where protein side chains usually come close to the heme. Hence this new site must expand somehow, and it does so in two directions. The upper half of the heme moves away to the left (*b* in Fig. 1, *a* in Fig. 2), nudging Asn 102β as it does so (*c* in Fig. 1) , while the root of Gln 28β is forced to the right and down (*a* in Fig. 1). Virtually no other disturbances are detectable; the amino acid replacement seems to have no effect on the α-subunit or on the sensitive $\alpha_1\beta_2$ subunit contact (left-hand border of Figs. 1 and 2) in this crystal form. However, in the other, fully oxygenated form, where subunit interfaces are somewhat changed, the disturbance of Asn 102β may have some significance. This residue forms the only hydrogen bond to cross the $\alpha_1\beta_2$ contact in oxyhemoglobin (1), but the absence of any alteration in the subunit dissociation constant for the oxy form of the mutant (6) argues that any effect on the strength of this bond must be small.

DISCUSSION

Though the stereochemical changes observed here are simple, the situation in vivo is probably not. Within the red cell, there must be at least three

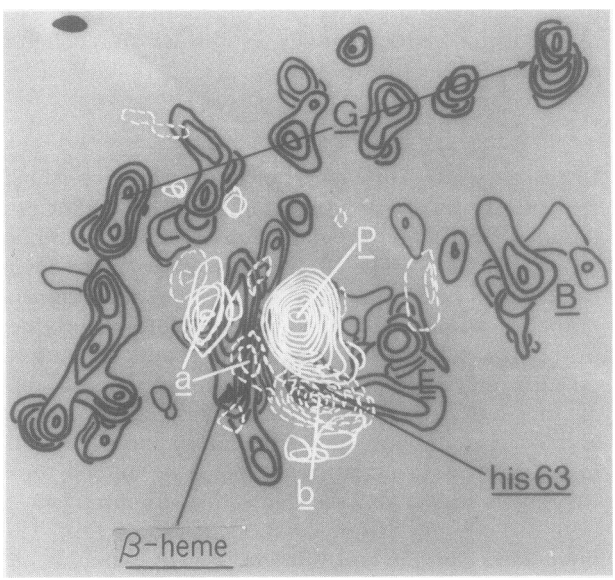

FIGURE 2 Sections $y = -5$ to -9 of the difference map, immediately below the region shown in Fig. 1. The total positive density in peak *P* (Figs. 1 and 2) is +29 electrons, while that in negative peak *b* is −10 electrons (removed from this position when His 63 moves into peak *P*). The difference, +19 electrons, neatly accounts for +7 electrons on replacing Leu with Gln and +10 electrons due to the addition of the water molecule. If the crystal were exposed to air, oxygen would bind at the β heme, just below peak *P*.

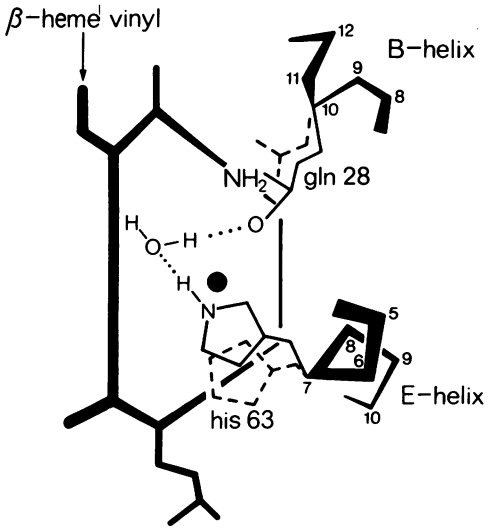

FIGURE 3 Stereochemistry at Hb St. Louis. Schematic diagram of the effect of the amino acid replacement, from the same viewpoint as Figs. 1 and 2 (placed one upon the other to show the whole mutation site). Solid lines show the configurations of His 63β, Leu 28β, and the helices to which they are attached, in normal Hb A. The broken lines show the changes indicated by the mutant difference map. A water molecule is hydrogen bonded (dots) between the new Gln 28 and His 63. The nearly vertical heme group (a square-planar complex with an iron atom at its centre) is seen in perspective to the left of these residues.

states of the Hb St. Louis β-hemes; a deoxy state (as observed here; no ligand), which can bind oxygen to give an oxy state, which is oxidized quickly and irreversibly to produce a metheme. The optical spectrum of whole blood containing Hb St. Louis shows that most of the mutant β chains are indeed metheme, while measurements on the pure reduced protein show that it undergoes conversion to this form very rapidly (6). Though only the deoxy state is shown here, a qualitative explanation for metheme formation may rest on the fact that heme exposed to water is quickly oxidized (8). The aqueous microenvironment shown could easily have a similar effect. Alternatively, the extra hydrogen-bonding capacity of the mutant heme pocket may stabilize bound oxygen as the superoxide radical, and by this means speed up oxidation (9). In any case, oxidation introduces another water molecule as heme ligand, probably without displacing the one at *P*. With two water molecules and a glutamine side chain buried in the core of the protein, unfolding and denaturation should occur relatively rapidly. This effect is observed (6) as an increased instability to temperature, and formation of Heinz bodies in vivo. Hemichrome Hb St. Louis (in which His 63β acts as the heme ligand) probably forms as a result of this structural instability, though the detailed mechanism is unknown.

The high affinity and low cooperativity of Hb St. Louis as it exists in the blood are almost certainly because its β chains are in the met state. In structural terms these two met-state chains closely resemble oxygenated subunits; hence the molecule appears always at least half saturated, unable to utilize its full working range of ligand saturation or affinity. Cooperativity is thus decreased. If the hemoglobin is fully reduced in vitro, then in the interval before reoxidation of the β hemes, almost normal affinity and cooperativity are observed (6). This argues against the importance of the mutation's more subtle structural consequences, such as displacement of the heme and residue Asn 102β.

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