Acta Cryst. (1994). D**50**, 283–289

1.70 Å Resolution Structure of Myoglobin from Yellowfin Tuna. An Example of a Myoglobin Lacking the *D* Helix

BY GEORGE I. BIRNBAUM,* STEPHEN V. EVANS, MARIA PRZYBYLSKA AND DAVID R. ROSE†

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

(*Received* 15 *July* 1993; *accepted* 20 *December* 1993)

Abstract

The crystal structure of metmyoglobin from yellowfin tuna (*Thunnus albacares*) has been determined by molecular replacement methods and refined to a conventional *R* factor of 0.177 for all observed reflections in the range of 6.0–1.70 Å resolution. Like other myoglobins for which a high-resolution structure is available, the polypeptide chain is organized into several helices that cooperate to form a hydrophobic pocket into which the heme prosthetic group is non-covalently bound; however, the *D* helix observed in other myoglobins is absent in myoglobin from yellowfin tuna and has been replaced with a random coil. As well, the *A* helix has a pronounced kink due to the presence of Pro16. The differences in structure between this and sperm whale myoglobin can be correlated with their reported dioxygen affinity and dissociation. The structure is in agreement with reported fluorescence data which show an increased Trp14···heme distance in yellowfin tuna compared to sperm whale myoglobin.

Introduction

Myoglobin functions in muscle tissue as an oxygen storage protein and serves to provide a diffusion gradient to absorb dioxygen from the blood into the cytoplasm where it subsequently can be utilized in the mitochondria. Few myoglobin structures from different species have been determined to high resolution. Myoglobin from sperm whale (SW, R = 0.177 at 2.0 Å resolution)‡ was the first protein for which a complete three-dimensional structure was determined (Kendrew *et al.*, 1958, 1960; Takano, 1977). More recently, high-resolution studies have been reported for horse heart (HH, R = 0.155 at 1.9 Å resolution) myoglobin (Evans & Brayer, 1988, 1990), myoglobin from the mollusc *Aplysia limacina* (Bolognesi *et al.*, 1989), and myoglobin from pig (Smerdon *et al.*, 1990; Oldfield *et al.*, 1992). Myoglobin from yellowfin tuna (*Thunnus albacares*, YFT) consists of a single polypeptide chain of 146 amino acids and a non-covalently bound protoporphyrin IX heme prosthetic group (Watts, Rice & Brown, 1980).

Many studies have focused on the oxygen-binding properties of the heme prosthetic group and the effect of the surrounding globin (Shikama, 1985). Of particular interest is the mechanism and exact path by which dioxygen enters and exits the heme cavity (Case & Karplus, 1979; Ringe, Petsko, Kerr & Ortiz de Montellano, 1984; Frauenfelder & Wolynes, 1985). YFT myoglobin is an ideal candidate to contribute to these investigations as it has been suspected of containing structural motifs that are significantly different from myoglobins of known structure, precisely at the predicted location of dioxygen entry to and exit from the heme pocket (Bismuto, Colonna, Savy & Irace, 1987). Further, physical studies have indicated that YFT myoglobin as a whole is less stable to denaturing conditions than mammalian myoglobins (Fosmire & Brown, 1976).

The crystal structure of YFT myoglobin has been determined previously by molecular replacement methods to 6 Å resolution by Lattman, Nockolds, Kretsinger & Love (1971), who concluded that the protein displayed the common myoglobin fold and that some differences from SW myoglobin were evident. The present study was undertaken in order to provide a firm structural basis for interpretation of the observed behavior of YFT myoglobin.

Experimental

Deep-red crystals of YFT metmyoglobin were obtained from hanging-drop vapor diffusion in a double-cell apparatus (Przybylska, 1989), using 2.6 *M* $(NH_4)_2SO_4$ at pH 7.0 as precipitant. Data were collected from a single crystal belonging to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 44.50, b = 72.32, c = 52.22 Å, with Z = 4. A Rigaku RU-200 generator with a Cu anode

* To whom correspondence should be addressed.

† Current address: Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9.

‡ The abbreviations used are: SW, sperm whale; HH, horse heart; YFT, yellowfin tuna.

ISSN 0907-4449

was operated at 40 kV and 150 mA, and two SDMS area detectors were positioned at −30 and 50° where 114 275 reflections were collected in eight sweeps. These reflections were merged to 17 376 unique reflections (96% complete to 2.0 Å resolution, and 87% complete to 1.70 Å resolution) with R_{sym} = 0.058. The data were corrected for Lorentz and polarization effects but not explicitly for absorption. A Wilson plot yielded an overall isotropic temperature factor of 28 Å^2.

The starting model was the structure of SW myoglobin (Takano, 1977) obtained from the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977). Corresponding amino acids that differed from those of YFT myoglobin were changed to glycine. The starting orientation was obtained using the Crowther fast-rotation function (Crowther, 1972) with a resolution of 10–3.5 Å that yielded an unambiguous peak 2.5 times larger than the standard deviation of the map. This same peak was also observed for other ranges of resolution, although it was not always the highest peak. The correct translation was found using the program *BRUTE* (Fujinaga & Read, 1987), which consistently showed the same solution for all ranges of resolution. The highest correlation that was finally observed was 0.41 for data from the 10–4.5 Å resolution range.

After an initial round of manual intervention where the correct sequence for YFT myoglobin was imposed on the starting structure, refinement of the starting model was initiated with *XPLOR* (Brünger, Kuriyan & Karplus, 1987), where simulated annealing was carried out in a number of steps using all data within the defined resolution ranges. Firstly, 4060 reflections between 5 and 2.3 Å were used in a 'fast' cooling from 2000 to 300 K, with 100 cycles of minimization, which reduced the R factor from 0.392 to 0.249. The resolution range was expanded to 6–2.3 Å (5541 reflections) for four cycles of simulated annealing; the final cycle began at 3000 K and concluded with minimization and individual B-factor refinement. This lowered the R factor from 0.284 to 0.235 for all data and 0.227 for the 5015 reflections with $I > 3\sigma(I)$.

All further refinement was carried out with the program *PROLSQ* (Hendrickson & Konnert, 1981), which was alternated with rounds of manual intervention using the program *FRODO* (Jones, 1978) to examine electron-density maps with coefficients ($|F_o| - |F_c|$) and ($2|F_o| - |F_c|$). The resolution was increased in stages during this procedure, and different electron-density peaks with appropriate hydrogen-bonding geometry were assigned as water molecules, until convergence was reached after 12 such rounds. After each round, water molecules that had a refined temperature factor greater than 70 Å^2 were omitted from further calculation. The final model consists of 144 amino-acid residues (the two carboxyl terminal residues of the polypeptide chain were not visible in the electron-density maps and were excluded from refinement), the heme group and 81 water molecules for a total of 1207 atoms. All side chains that were included in the refinement displayed appropriate electron density, with no evidence of significant disorder. The final R factor for the 16 202 reflections from 6 to 1.70 Å resolution with $I > 1\sigma(I)$ was 0.229 (no water molecules) and 0.177 (with water molecules included).

Table 1. *Deviations from ideal stereochemistry for YFT myoglobin*

Refinement parameter	R.m.s. deviation from ideal value	Refinement restraint weighting value
Bond distances (Å)	0.019	0.025
Angle distances (Å)	0.041	0.050
Planar 1–4 distances (Å)	0.044	0.050
Planes (Å)	0.026	0.030
Chiral volumes (Å^3)	0.157	0.100
ω bond angles (°)	1.8	10.0
Single torsion contacts (Å)	0.201	0.200
Multiple torsion contacts (Å)	0.207	0.200
Hydrogen-bond distances (Å)	0.165	0.200

The stereochemical parameters from the last cycle of refinement are presented in Table 1 and show good agreement with literature values. A plot of R factor *versus* $\sin\theta/\lambda$ (after Luzzati, 1952) shows that the upper limit for the average coordinate error for all atoms in the structure is approximately 0.20 Å (positional errors for atoms located in the well defined center of the molecule would be expected to be much lower).

Results and discussion

Overall structure

The conformations of the α backbone, heme moiety, and the heme ligand His93 of YFT myoglobin are presented in Fig. 1(*a*), and of all main-chain, side-chain and heme atoms in Fig. 1(*b*). [All figures were generated using programs in the *SETOR* program package (Evans, 1993)]. The overall structure of YFT myoglobin is similar to other structures in the globin family (Bashford, Chothia & Lesk, 1987), where six α-helices and one 3_{10} helix collectively form a hydrophobic pocket in which the heme prosthetic group is non-covalently bound. Of 146 residues, 99 (68%) are involved in helices; this compares to 42–46% predicted on the basis of ORD measurements (Fosmire & Brown, 1976). Fig. 2 is a plot of φ–ψ main-chain dihedral angles (Ramakrishnan & Ramachandran, 1965); it shows that most residues have values that cluster about those expected for an α-helical structure (McGregor, Islam & Sternberg, 1987). The distribution of side-chain dihedral angles

is presented in Fig. 3. As found for other helical proteins (McGregor *et al.*, 1987; Ponder & Richards, 1987), the χ_1 angles in YFT myoglobin show a strong aversion to the g^- conformation (60°) and favor the g^+ and t conformations (−60 and 180°, respectively).

Comparison with other myoglobins

The amino-acid numbering scheme for YFT myoglobin is based on the alignment of its primary sequence with that of SW myoglobin, Fig. 4. The two proteins have sequence identity of 45% and a functional homology of 60% (Devereux, Haeberli & Smithies, 1984), and there are some non-conservative differences in sequence that are reflected in the structure. Most notable is the absence of the *D* helix usually observed in other myoglobins (residues 51–57 in SW myoglobin). What would have been the *D* helix in YFT myoglobin has 'opened' into a random coil (Fig. 5) interspersed with three tight turns. Interestingly, this group of turns is composed of one representative each of types I, II and III (Richardson, 1981; Table 2). The loss of the *D* helix had been predicted by Bismuto *et al.* (1985) on the basis that the two substitutions E52Q and K56A precluded the formation of a salt bridge from residue 52 to residue 56 and a hydrogen bond from residue 26 to residue 56, which had been thought to be necessary to stabilize the *D* helix. The other predictions made by Bismuto *et al.* (1985) of a short *A* helix, a break in the *E* helix, a shorter *F* helix and a break in the *H* helix, are not observed, although there is a pronounced kink in the *A* helix caused by the substitution of proline for lysine at residue 16.

A superposition of corresponding main-chain atoms for the YFT and SW proteins* gave an r.m.s. deviation of 1.32 Å. Table 3 shows the results from the superposition of individual helices. Aside from the changes in the *A* and *D* helices already mentioned, the deviations within individual helices are

* The coordinates of SW myoglobin used for comparison were from the 1989 update to the Protein Data Bank, code 4MBN.

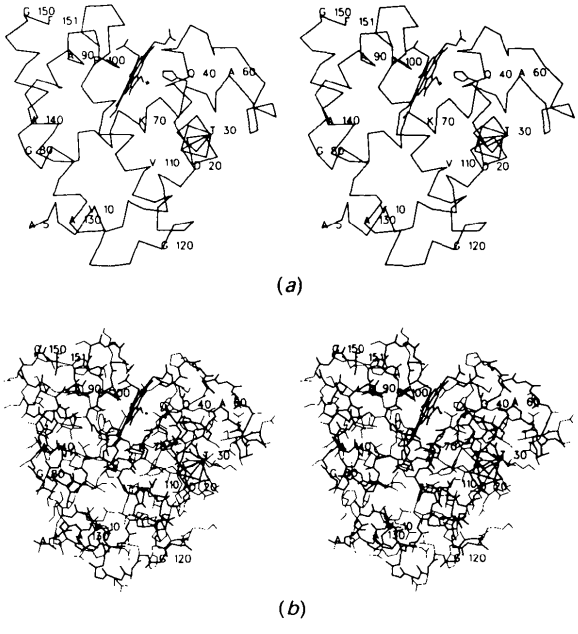

Fig. 1. Stereo drawings of (*a*) α backbone, heme group and heme ligand His93, and (*b*) all non-H atoms of YFT myoglobin. The main-chain atoms are drawn with thick lines while side-chain atoms are drawn with thin lines. The terminal residues and every tenth residue are labeled with their respective one-letter amino-acid codes.

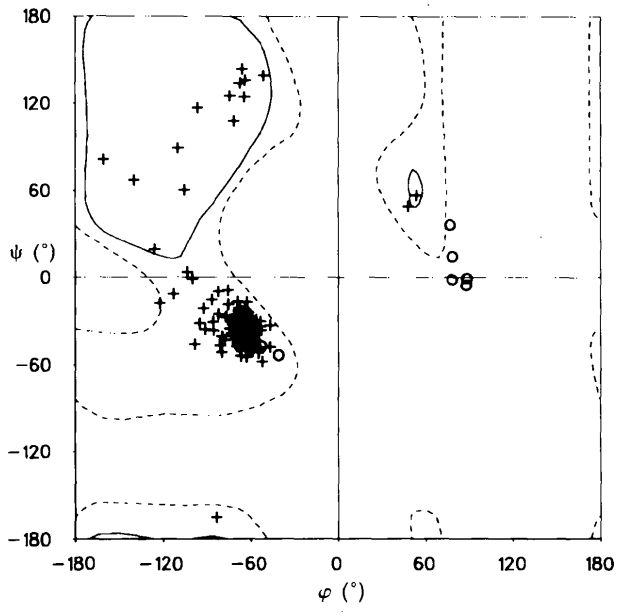

Fig. 2. Plot of φ–ψ main-chain dihedral angles with glycine residues denoted by circles (Ramakrishnan & Ramachandran, 1965). No residues fall significantly outside the allowed regions of the plot.

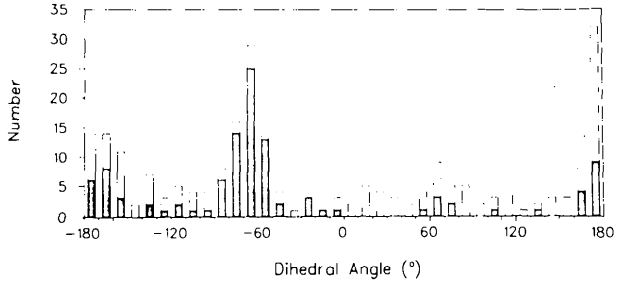

Fig. 3. Distribution of all side-chain dihedral angles which are expected to assume a staggered conformation in YFT myoglobin, shown as open bars. The filled bars represent the fraction of entries that are made up of χ_1 dihedral angles.

much smaller than the overall deviation, and so most of the changes in tertiary structure between the two proteins must result from a repositioning of the helices, rather than from movement of individual residues.

Fig. 6 shows the mean isotropic temperature factors for main-chain and side-chain atoms over the course of the polypeptide chain. In a pattern also observed in other myoglobins, the temperature factors are highest at the amino and carboxy termini, and at the interhelical regions – particularly the *EF* loop. The temperature factors for residues corresponding to the *D* helix of other myoglobins (residues 51–57) are not significantly higher than the average for the molecule, and the chain lies in well defined electron density, Fig. 7. As found for HH myoglobin, the temperature factors of the side chains alternate between values close to their parent main-chain atoms for buried residues and values 10–15 Å^2 higher for residues exposed to the solvent (Evans & Brayer, 1990). The temperature factors for the water molecules are on the same order as those of the polypeptide chain, ranging from 13.8 Å^2 for the water molecule occupying the sixth coordination site of the heme Fe atom, to 69.2 Å^2.

It is interesting to note that the regions of the primary sequence most highly conserved between SW and YFT myoglobin are those that are in closest contact with the heme group. There are only two stretches of the primary sequence alignment that maintain 80% or better homology for 10 or more consecutive residues, namely from residue 29 to 39 and from residue 64 to 101. These two portions of the polypeptide chain encompass the latter half of the *B* helix and the entire length of the *E* and *F* helices (including the distal and proximal His64 and His93) and account for almost all of the direct contacts between globin and heme. This corresponds to earlier studies which show the heme pocket of myoglobins to be a highly conserved feature (Evans & Brayer, 1988). In contrast, there are two areas that show no sequence homology for ten consecutive residues: from residues 54 to 63 and from residues 131 to 140. Neither of these regions is in direct contact with the heme group, but residues 54 to 63 make up most of what is the *D* helix in SW myoglobin and what has been found in the present study to be a random coil in YFT myoglobin. Significantly, the *D* helix region is close to the suspected area of dioxygen entry to and exit from the heme cavity (Case & Karplus, 1979; Ringe *et al.*, 1984; Frauenfelder & Wolynes, 1985). These observations may explain the fact that dioxygen dissociates three times faster from YFT than from SW myoglobin, although oxygen affinities for the two proteins are identical

Table 2. *Tight turns observed in the D-region of YFT myoglobin*

Residues	φ_2 (°)	ψ_2 (°)	φ_3 (°)	ψ_3 (°)	Turn type
51–54	−58.5 (−60)*	−35.1 (−30)	−62.0 (−60)	−24.1 (−30)	III
52–55	−62.0 (−60)	−24.1 (−30)	−100.0 (−90)	−1.3 (0)	I
55–58	−67.2 (−60)	134.4 (120)	78.0 (90)	−1.6 (0)	II

* Numbers in parentheses are the approximate values expected for the designated turn type.

Table 3. *Comparison of helices in YFT myoglobin and SW myoglobin*

Helix	YFT	SW	R.m.s. (Å)
A	Asp6 Ala19	Glu6 Ala19	1.16
B	Tyr21 Glu35	Val21 Ser35	0.30
C	Pro37 Leu42	Pro37 Lys42	0.49
E	Ala60 Lys77	Asp60 Lys77	0.40
F	Ile85 His93	Glu85 His93	0.28
G	Ile101 Glu117	Ile101 Ser117	0.49
H	Gly126 Asn145	Gly124 Ala143	0.83

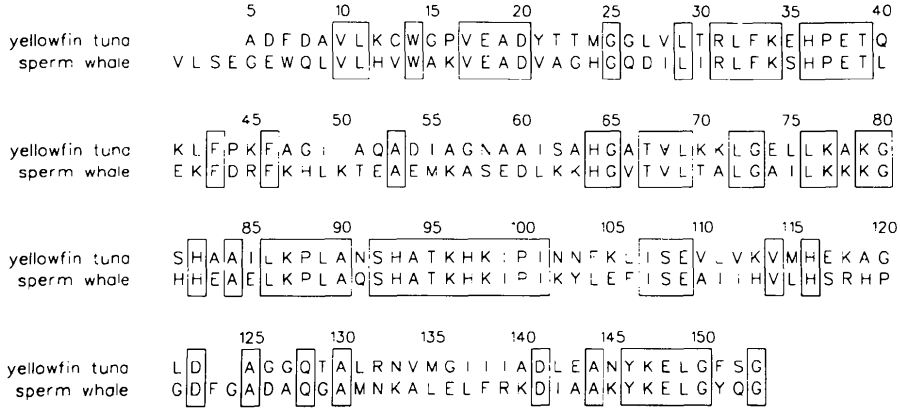

Fig. 4. Alignment of the primary sequences of YFT and SW myoglobin.

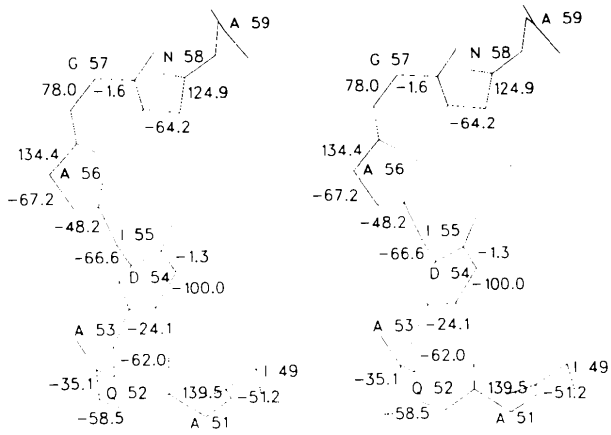

Fig. 5. Stereoview of the main-chain atoms of residues in YFT myoglobin that make up what is usually seen as the *D* helix in other myoglobins. Residues are labeled with their respective one-letter codes. Also shown are all the main-chain φ ψ dihedral angles ( ). The dashed lines represent the hydrogen bonds involved in the three observed tight turns.

within experimental error (Livingston, Watts & Brown, 1986). This is because the oxygen affinity of the heme iron would be most highly dependent on its surroundings which are conserved between the species, while the dissociation through the globin would be more facile in YFT myoglobin with its more flexible *D*-region random coil.

A stereoview of the heme group and its associated electron density is presented in Fig. 8. The four pyrrole N atoms do not deviate by more than 0.01 Å from their least-squares plane, but the heme-iron atom is displaced by 0.09 Å from this plane toward the heme ligand His93. This is in agreement with the values found for HH myoglobin and pig myoglobin. This contrasts with the value of 0.40 Å first reported for the high-resolution SW myoglobin structure (Takano, 1977), although coordinates from more recent refinements of SW myoglobin show a heme-iron displacement closer to 0.15 Å. As found for SW and HH myoglobin, there is a water molecule at the

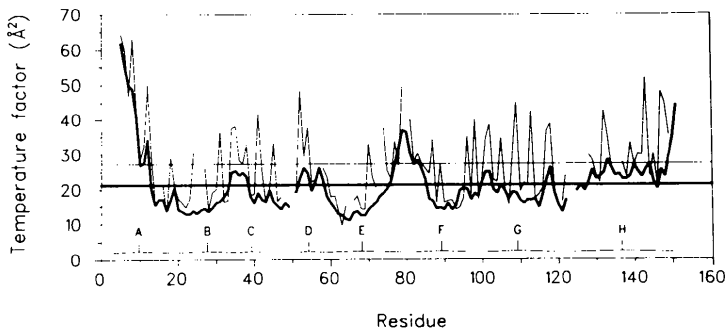

Fig. 6. Mean isotropic temperature factors for main-chain (thick line) and side-chain (thin line) atoms of YFT myoglobin. The corresponding two horizontal lines represent the observed overall mean values.

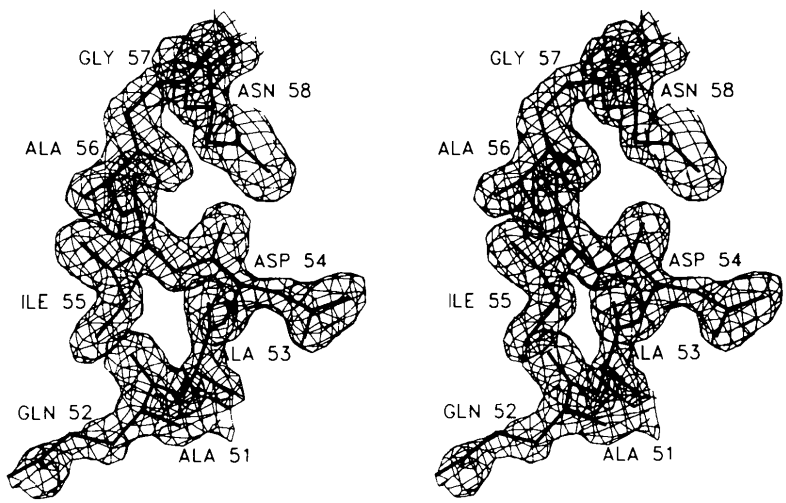

Fig. 7. Stereoview of residues 51-57 in YFT myoglobin. Despite not being part of a regular helix, as in other myoglobins, these residues lie in well defined electron density.

sixth coordination site on the heme-iron atom that also forms a hydrogen bond with the distal histidine. No such water molecule was observed in pig myoglobin, which was ascribed to the fact that the crystals were grown at pH 7.1 while SW and HH myoglobins were grown at more acidic pH; however, YFT myoglobin crystals were grown at pH 7.0. The heme propionate groups are oriented similarly to those in HH and SW myoglobin.

The hydrogen bonds involving side-chain atoms observed in YFT myoglobin are presented in Tables 4 and 5. Each hydrogen bond was required to have a donor atom–H atom–acceptor atom angle greater than 120°, and an H atom–acceptor atom distance of less than 2.4 Å where the acceptor was an O atom, and less than 2.5 Å where the acceptor was a N atom. H atoms were placed in calculated positions, and those that could not be positioned unambiguously (such as those of hydroxyl groups) were placed in optimal geometry with respect to each potential acceptor. As in other helical proteins, most of these interactions are between residues separated by five or fewer amino acids on the polypeptide chain (corresponding to one turn of a helix). On the basis of primary structure Watts *et al.* (1980) had predicted three globin salt bridges and two globin intersegmental hydrogen bonds; however, there are actually seven globin salt bridges, and nine hydrogen bonds are observed between different segments of the molecule. The number of intersegmental hydrogen bonds and salt bridges observed in YFT myoglobin is similar to both the HH and SW myoglobins. The interactions with the heme propionate groups for YFT myoglobin are identical to those in HH myoglobin and involve Lys45 (Arg45 in SW myoglobin), Ser92 and His97. Both SW and HH myoglobin display similar packing in the unit cell and both have a sulfate ion bound at the close approach of the

Table 4. *Hydrogen bonds involving side-chain atoms with unambiguous* H-*atom positions*

Donor		Acceptor		*D*···*A* (Å)	*D*···*A*—C (°)
Trp14	NE1—HE1	Tyr21	OH	3.04	119
Thr23	N—H	Asp20	OD1	3.09	112
Arg31*	NE—HE	Glu35	OE1	2.65	120
Arg31*	NH1—HH12	Glu117	OE1	2.89	114
Arg31*	NH2—HH22	Glu117	OE2	3.18	103
His36	NE2—HE2	Glu109	OE1	2.80	151
Glu38	N—H	Glu38	OE1	2.74	101
Gln40	OE1—HE11	Phe46	O	2.84	143
Gln40	OE1—HE12	Ile49	O	3.01	127
Ile61	N—H	Asn58	OD1	3.07	138
His82	NE2—HE2	Asp141	OD2	2.65	129
His93	ND1—HD1	Leu89	O	2.85	142
His93	ND1—HD1	Ser92	OG	3.04	145
His97	NE2—HE2	Hem154	O1A	2.82	129
Asn103	OD1—HD11	Pro100	O	3.09	121
His116	ND1—HD1	Leu121	O	2.81	165
Gly127	N—H	Asp122	OD1	2.95	165
Asn133	OD1—HD11	Asp6	OD1	2.73	160

* Charged residue interaction.

Table 5. *Hydrogen bonds involving side-chain atoms with ambiguous* H-*atom positions*

Donor		Acceptor		*D*···*A* (Å)	*D*···*A*—C ( )
Lys12*	NZ—HZ1	Asp8	O	2.86	137
Thr23	OG1—HG1	Asp20	OD2	2.66	109
Thr30	OG1—HG1	Gly26	O	2.82	135
Thr39	OG1—HG1	His36	O	2.61	167
Lys45*	NZ—HZ1	Hem154	O1D	2.86	96
Lys45*	NZ—HZ1	Hem154	O2D	2.98	90
Ser62	OG—HG	Asn58	O	3.14	137
Thr67	OG1—HG1	Ala63	O	3.29	141
Lys71*	NZ—HZ1	Glu74	OE1	2.90	104
Lys77*	NZ—HZ1	Glu18	OE2	2.80	111
Ser92	OG—HG	Leu89	O	3.18	118
Ser92	OG—HG	Hem154	O2A	2.61	130
Thr95	OG1—HG1	Asn91	O	2.75	140
Ser108	OG—HG	Phe104	O	2.67	154
Lys113*	NZ—HZ1	Glu109	O	2.78	111
Thr129	OG1—HG1	Ala125	O	3.32	152
Tyr146	OH—HH	Ile99	O	2.66	148

* Charged residue interaction.

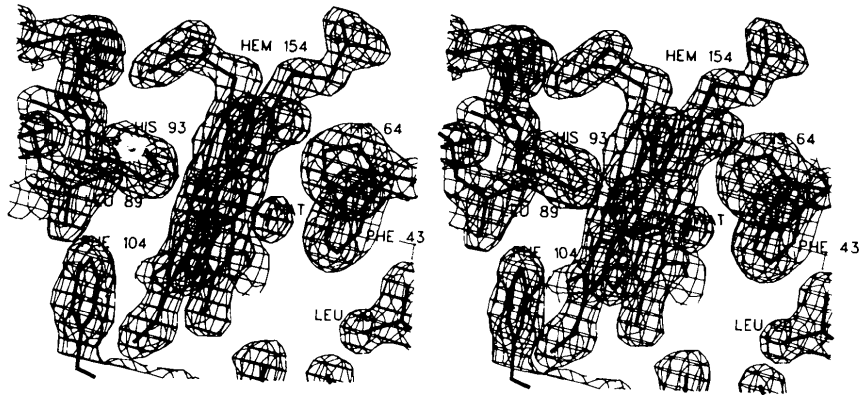

Fig. 8. Stereoview of the heme group and its distal and proximal histidine groups, and their corresponding electron density. The heme iron is displaced by 0.09 Å out of the mean plane of the pyrrole N atoms toward the heme ligand His93.

N-terminal regions of the *E* helix and the *D* helix of an adjacent molecule. No sulfate ion is observed in YFT myoglobin, which does not have a *D* helix and also packs differently from HH and SW myoglobin.

A final point concerns the single tryptophan residue in YFT myoglobin that has been utilized in fluorescence studies both in solution (Willis, Szabo, Zuker, Ridgeway & Alpert, 1990) and in the crystal (Willis, Szabo & Krajcarski, 1991), where it was shown that the Trp14 fluorescence lifetime was longer in YFT than SW myoglobin. This indicated that there is a larger distance between Trp14 and the heme group (which quenches the Trp fluorescence) in YFT compared to SW myoglobin. This result is in agreement with the crystal structure described in the present study and implies that the two myoglobins maintain their respective structures whether in the crystal or in solution.

Coordinates and structure factors for YFT myoglobin have been submitted for deposition in the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977).* This is NRC publication number 37376.

We thank Mrs R. To for preparing single crystals of YFT myoglobin, Mrs M. E. Pippy for computational assistance, Dr M. Zuker for the least-squares superpositions and the sequence alignments, and Drs A. G. Szabo, K. J. Willis, F. R. Ahmed and D. Ringe for helpful discussions.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1MYT, R1MYTSF). Free copies may be obtained through the Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Supplementary Publication No. SUP 37111). A list of deposited data is given at the end of this issue.

References

Bashford, D., Chothia, C. & Lesk, A. M. (1987). *J. Mol. Biol.* **196**, 199–216.

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.

Bismuto, E., Colonna, G., Savy, F. & Irace, G. (1985). *Int. J. Pept. Protein Res.* **26**, 195–207.

Bolognesi, M., Onesti, S., Gatti, G., Coda, A., Ascenzi, P. & Brunori, M. (1989). *J. Mol. Biol.* **205**, 529–544.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). *Science*, **235**, 458–460.

Case, D. A. & Karplus, M. (1979). *J. Mol. Biol.* **132**, 343–368.

Crowther, R. A. (1972). *The Molecular Replacement Method*, edited by M. G. Rossmann, pp. 173–178. New York: Gordon & Breach.

Devereux, J., Haeberli, P. & Smithies, O. (1984). *Nucleic Acids Res.* **12**, 387–395.

Evans, S. V. (1993). *J. Mol. Graphics* **11**, 134–138.

Evans, S. V. & Brayer, G. D. (1988). *J. Biol. Chem.* **263**, 4263–4268.

Evans, S. V. & Brayer, G. D. (1990). *J. Mol. Biol.* **213**, 885–897.

Fosmire, G. J. & Brown, W. D. (1976). *Comp. Biochem. Physiol. B*, **55**, 293–299.

Frauenfelder, H. & Wolynes, P. G. (1985). *Science*, **229**, 337–345.

Fujinaga, M. & Read, R. J. (1987). *J. Appl. Cryst.* **20**, 517–521.

Hendrickson, W. A. & Konnert, J. H. (1981). *International Symposium on Biomolecular Structure*, edited by R. Srinivasan, pp. 43–57. Oxford: Pergamon Press.

Jones, T. A. (1978). *J. Appl. Cryst.* **11**, 268–272.

Kendrew, J. C., Bodo, G., Dintzis, H. M., Parrish, R. G., Wyckoff, H. & Phillips, D. C. (1958). *Nature (London)*, **181**, 662–666.

Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C. & Shore, V. C. (1960). *Nature (London)*, **185**, 422–427.

Lattman, E. E., Nockolds, C. E., Kretsinger, R. H. & Love, W. E. (1971). *J. Mol. Biol.* **60**, 271–277.

Livingston, D. J., Watts, D. A. & Brown, W. D. (1986). *Arch. Biochem. Biophys.* **249**, 106–115.

Luzzati, V. (1952). *Acta Cryst.* **5**, 802–810.

McGregor, M. J., Islam, S. A. & Sternberg, M. J. E. (1987). *J. Mol. Biol.* **198**, 295–310.

Oldfield, T. J., Smerdon, S. J., Dauter, Z., Petratos, K., Wilson, K. S. & Wilkinson, A. J. (1992). *Biochemistry*, **31**, 8732–8739.

Ponder, J. W. & Richards, F. M. (1987). *J. Mol. Biol.* **193**, 775–791.

Przybylska, M. (1989). *J. Appl. Cryst.* **22**, 115–118.

Ramakrishnan, C. & Ramachandran, G. N. (1965). *Biophys. J.* **5**, 909–933.

Richardson, J. S. (1981). *Adv. Protein Chem.* **34**, 167–339.

Ringe, D., Petsko, G. A., Kerr, D. E. & Ortiz de Montellano, P. R. (1984). *Biochemistry*, **23**, 2–4.

Takano, T. (1977). *J. Mol. Biol.* **110**, 537–568.

Shikama, K. (1985). *Experientia*, **41**, 701–706.

Smerdon, S. J., Oldfield, T. J., Dodson, E. J., Dodson, G. G., Hubbard, R. E. & Wilkinson, A. J. (1990). *Acta Cryst.* **B46**, 370–377.

Watts, D. A., Rice, R. H. & Brown, W. D. (1980). *J. Biol. Chem.* **255**, 10916–10924.

Willis, K. J., Szabo, A. G. & Krajcarski, D. T. (1991). *J. Am. Chem. Soc.* **113**, 2000–2002.

Willis, K. J., Szabo, A. G., Zuker, M., Ridgeway, J. M. & Alpert, B. (1990). *Biochemistry*, **29**, 5270–5275.