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Three-Dimensional Structure of a Hemichrome Hemoglobin from *Caudina arenicola*

BY DAVID T. MITCHELL, STEPHEN R. ERNST, WEI-XIN WU AND MARVIN L. HACKERT*

Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712, USA

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Abstract

The structure of a monomeric hemichrome form of an invertebrate hemoglobin, Hb-C chain, from *Caudina arenicola* has been refined to an R value of 0.16 using the data from 5.0 to 2.5 Å resolution ($R = 0.21$ from 10.0 to 2.5 Å resolution). Hb-C crystallizes in space group $P2_1$ with cell constants $a = 45.74$, $b = 45.23$ and $c = 40.92$ Å and $\beta = 104.4°$ with two monomers packed in the unit cell ($V_m = 2.34$ Å^3 Da^{-1}). The phases were determined by the multiple isomorphous replacement method with Hg^{2+} the major derivative. The structure consists of 157 amino acids with N- and C-terminal regions and eight α-helices forming a heme pocket. The unique feature of this structure is the hemichrome form with the proximal and distal histidines coordinated to the heme Fe atom, which is nearly in the plane of the porphyrin ring. A total of 111 solvent molecules were added to the structure using difference density peaks of at least 3σ over background. Interestingly, all the heme groups present in the crystal are nearly coplanar.

Introduction

Hemoglobin is the most widely distributed transport molecule, serving as an oxygen carrier in nearly all vertebrate organisms. In addition to supplying the tissues with oxygen, human hemoglobin removes carbon dioxide produced by metabolism and regulates the pH of the blood. The three-dimensional structure and biochemical properties such as ligand-induced change, allosterism and cooperativity of human hemoglobin are discussed in textbooks as classical studies. The cooperative binding of oxygen by the vertebrate hemoglobin heterotetramer $(\alpha\beta)_2$ is accomplished by structural rearrangements between all four subunits.

Less is known about the hemoglobins of invertebrate organisms due in part to the uneven distribution of invertebrate species that possess hemoglobin. Ligand-binding properties of hemoglobins from invertebrate phyla frequently have properties that differ from vertebrate hemoglobins. The cooperative binding of oxygen is not a common property to all invertebrate hemoglobins. Studies have shown that invertebrate hemoglobins exist as monomers, dimers, tetramers or larger aggregates depending on the presence or absence of ligands. Three-dimensional structures of only a few invertebrate hemoglobins have been determined. These include the monomeric hemoglobins from *Glycera dibranchiata* (Padlan & Love, 1974; Braden, Arents, Padlan & Love, 1994), and *Chironomus thummi thummi* (Huber, Epp, Steigmann & Formanck, 1971; Steigemann & Weber 1979), the dimeric and tetrameric forms from *Scapharca inaequivalvis* (clam) (Royer, Hendrickson & Chiancone, 1989; Royer, 1994), and the homotetrameric hemoglobin from the fat inn-keeper worm *Urechis caupo* (Kolatkar *et al.*, 1991). In each case there is conservation of tertiary structure with preservation of the 'myoglobin fold', but the quaternary structures seen in the *Scapharca* and *Urechis* hemoglobins differ markedly from that observed for vertebrate hemoglobins and from each other.

Caudina arenicola, a sea cucumber, is a member of the Echinoderm phylum that is thought to be closely related to vertebrates. The divergence is thought to have occurred about 500 million years ago, about the same time as the split of the vertebrate hemoglobin genes that code for the α and β chains. The sea cucumber burrows in the mud and pumps water into its coelomic cavity allowing for gas exchange. Blood in the coelomic cavity was found to contain seven different dimeric hemoglobins made from four different chains labelled *A*, *B*, *C* and *D* (Whitfill, 1973; Bonaventura & Kitto, 1973). Immunochemical methods have shown that the *A*, *B* and *C* chains are very similar with the *D* chain being more distinct (Dodds, 1977). Protein sequencing of the *C* and *D* chains showed that the two chains are only 65% identical (McDonald, Davidson & Kitto, 1992; Mauri, Omnaas, Davidson, Whitfill & Kitto, 1991). The oxy-, carbonmonoxy-, and cyanomet-liganded hemoglobin forms are dimeric, but the methemoglobins are monomeric at all observed protein concentrations. Deoxy forms of the *Caudina* hemoglobins are thought to exist as tetramers or higher oligomers. Homodimers of the individual chains were found to be slightly cooperative with respect to ligand binding, which is not the case for human α and β hemoglobin (Bonaventura, Bonaventura, Kitto, Brunori & Antonini, 1976). Heterodimers exhibit enhanced cooperativity of oxygen binding.

Hemichromes are heme proteins where both the fifth and sixth coordination sites of the heme Fe atom are

* Author to whom all correspondence should be addressed.

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bound to a nitrogenous base such as imidazole (Rachmilewitz, Peisach, Bradley & Blumberg, 1969). Hemichrome formation is an oxidation reaction that involves the conversion of a high-spin Fe^{2+} to a low-spin Fe^{3+} *via* an intermediate met hemoglobin form. This can be observed by electron paramagnetic resonance (EPR) or spectrophotmetrically by an absorption peak at 530–535 nm (Rachmilewitz *et al.*, 1969). Recently, two separate four-helix oligopeptides have been synthesized that are able to bind heme with characteristics similar to hemichrome hemoglobins (Choma *et al.*, 1994). Invertebrate and vertebrate hemoglobins can form hemichrome hemoglobins by a similar mechanism (Spagnuolo, Rinelli, Coletta, Chiancone & Ascoli, 1987) using reducing agents such as nitrite (Spagnuolo *et al.*, 1988). Hemichrome formation in human red blood cells can be the result of abnormalities in the ratio of α and β chains or in the enzyme systems that protect hemoglobins against oxidation (Dacie *et al.*, 1964). Studies on human hemoglobin tetramers as well as the individual chains have shown that the combination of unlike subunits can prevent hemichrome formation (Rachmilewitz, Peisach & Blumberg, 1971). Hemoglobin H (α-thalassemia) is a disease characterized by hemichrome formation related to an over abundance of β-chains (Dickerson & Geis, 1983). The β-chains assemble into β_4 molecules that have high oxygen affinity, do not bind oxygen cooperatively (Kurtz, Rollema & Baurer, 1981) and form hemichromes, causing the hemoglobin molecule to precipitate out of solution and form inclusion bodies in the cell, referred to as Heinz bodies (Dacie *et al.*, 1964). High-resolution X-ray crystallographic structures of human β_4 have been determined in the carbonmonoxy and deoxy states (Borgstahl, Rogers & Arnone, 1994*a*,*b*) that draw interesting conclusions about the stability of the β_4 molecule and the conversion of the human $(\alpha\beta)_2$ heterotetramer from the deoxy to oxy states, but there is no evidence of hemichrome Fe atom coordination in the deoxy structure.

The X-ray structure that we report for the *C*-chain hemoglobin from *Caudina arenicola*, hereafter referred to as Hb-C (not to be confused with the human Hb mutant Hb-C with E6K β), features a hemichrome coordination in a monomeric structure. This hemoglobin also has long N- and C-terminal extensions that lack secondary structure. The combination of cooperativity of oxygen binding and ligand-linked aggregation states in hemoglobins from an invertebrate organism closely related to vertebrates makes the study of hemoglobins from *Caudina arenicola* interesting from structural and biochemical points of view.

Materials and methods

Hemoglobin C (Hb-C) was purified from nucleated red blood cells of *Caudina arenicola* (Mauri *et al.*, 1991) and crystallized as previously described (Carson, Bowers, Kitto & Hackert, 1979). Native and derivative data sets were collected on a Syntex four-circle goniostat equipped with a Picker X-ray generator and a *KRISEL CONTROL* automation package. Intensity data were obtained using a modified ω-scan mode. The scan rate was constant at 1.5° min^{-1} with 1.5 min per reflection and a background-to-peak ratio of 0.4. Three standard reflections were measured after every 100 reflections to monitor crystal and instrument stability. Intensities were corrected for crystal decay and absorption corrections were applied using an empirical φ-scan approach. Intensities were further corrected for Lorentz and polarization effects. After corrections, the native data set was 84% complete to 2.5 Å resolution with intensities observed out to 2.1 Å resolution. Heavy-atom positions were determined by difference Patterson and Fourier analysis. The positions were refined and phases were calculated using the series of programs by Dr G. Petsko.

Crystallographic refinement of Hb-C used both the package of Konnert & Hendrickson (Hendrickson &

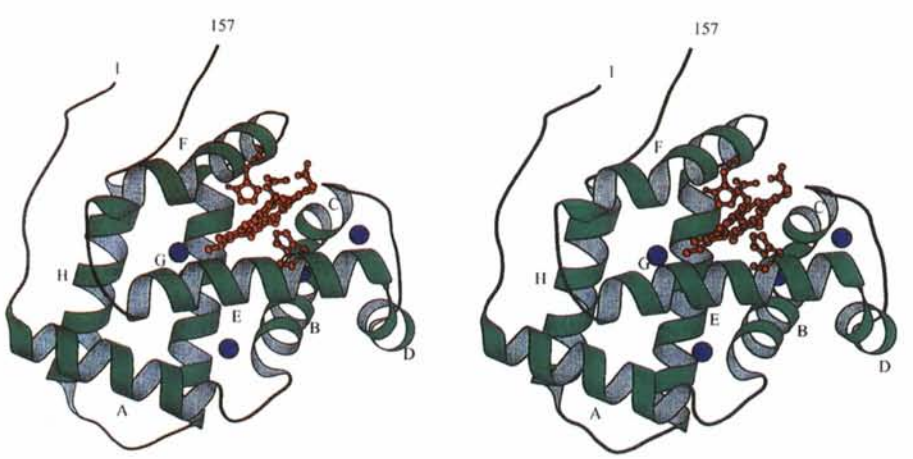

Fig. 1. Ribbon drawing (*MOLSCRIPT*, Kraulis, 1991) of the Hb-C monomer viewed nearly down the crystal *b* axis with the helices labelled. The heme group and proximal and distal histidines shown as ball-and-stick atoms. The four internal water molecules are shown as blue spheres.

Konnert, 1980) and the energy minimization and simulated annealing of *X-PLOR* Version 2.1 (Brünger, Kuriyan & Karplus, 1987). The final model was refined to 2.5 Å resolution using the 5.0 to 2.5 Å shell of data (4916 reflections). A typical round of *X-PLOR* refinement run on a Cray Y-MP 8/864 included energy minimization, simulated annealing and individual temperature-factor refinement. Once solvent molecules were added, no further simulated annealing was carried out. After each round of refinement $(2F_o - F_c)\alpha_c$ electron-density maps were calculated and manual rebuilding was carried out using *FRODO* (Jones, 1979) running on an Evans and Sutherland PS 390 or *O* (Jones, Zou, Cowan & Kjeldgaard, 1991) on a Silicon Graphics Crimson workstation.

The electron-density maps used in the final rounds of model building were NED (non-existent density) maps generated using the program *NEDFFT* (Kolatkar *et al.*, 1991), whereby the electron density is sequentially set to zero for a series of slabs over the asymmetric unit. Back transformation yields new phases (α_c') which are combined with the amplitude $(2wF_{\text{nat}} - F'_{\text{calc}})$ (Main, 1979) to minimize model bias in the electron density calculated for the modified slab and the process is repeated over the entire asymmetric unit. NED maps were typically calculated using data from 10.0 to 2.5 Å resolution. In addition, standard *OMIT* maps were calculated with *X-PLOR* to help position residues in the loop regions and the N and C termini.

Solvent molecules were selected from peaks greater than 3σ in NED and difference Fourier maps. Furthermore, to be included as a solvent molecule its density had to be well shaped and have at least one potential hydrogen-bond mate in either a side-chain or main-chain atom. If a solvent molecule was added and found to undergo a large shift in *X*, *Y* and *Z* position (greater than 1.0 Å in two directions) during refinement or assumed a large temperature factor (> 70 Å^2) it was removed. A total of 111 solvent molecules were added in three rounds of refinement. There are four internal solvent molecules (Fig. 1) while the rest appear to coat the outer surface of the protein structure.

Results

The needle-like crystals of Hb-C belong to space group $P2_1$ with $a = 45.74$, $b = 45.23$ Å and $c = 40.92$ Å and $\beta = 104.4°$ with one molecule in the asymmetric unit. Under plane-polarized light, these crystals appear colorless in select orientations when rotated about their needle (*b*) axis. This implies that all heme planes of the unit cell must be nearly parallel with the *b* axis and must be perpendicular to the direction of the plane-polarized light when they lose their characteristic dark red color. Fig. 1, which is a ribbon drawing of the Hb-C molecule, is shown nearly down the *b* axis of the crystal and indicates that the heme group is indeed parallel with the *b* axis.

The Hb-C structure was initially solved at 3.7 Å resolution by means of multiple isomorphous replacement techniques (MIR). Table 1 gives the data-collection statistics for the native and derivative data sets used in the structure determination while Table 2 reports the final heavy-atom positions. An electron-density map calculated at 3.7 Å resolution based on phases derived from these positions was used to build an initial model. Statistics on the heavy-atom structure factor (f_H) and lack of closure (E) for each derivative and the MIR mean figure of merit (FOM) as a function of resolution are shown in Table 3. By way of confirmation of this solution, an anomalous difference Patterson map calculated from native data using $(|F_h^+| - |F_h^-|)^2$ as coefficients has its largest peak within 1 Å of the predicted iron–iron vector derived from the final model (Carson, 1980).

The initial low-resolution map readily revealed eight helices of the globin structure. Unfortunately, lack of the amino-acid sequence and problems with the loop regions and the N and C terminal made it difficult to build a complete model. However, evidence of the hemichrome form of Hb-C was clear. The final model was arrived at by repeated rounds of refinement and model building. Once the amino-acid sequence was available and fitted to the map, 2.5 Å resolution data was incorporated in the *X-PLOR* refinement. The recent cDNA sequence for Hb-C (Thomas, 1994) corrected two errors in the previously

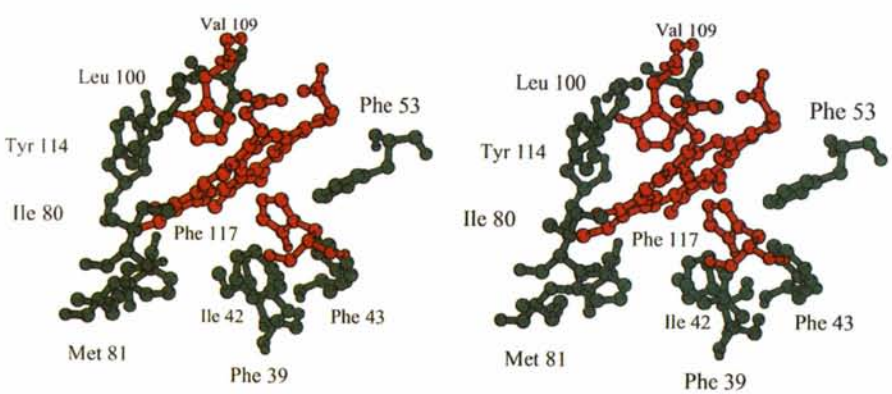

Fig. 2. Ball-and-stick model (*MOLSCRIPT*, Kraulis, 1991) of the heme region of Hb-C showing the large hydrophobic residues packed around the distal side of the heme group.

Table 1. *Data-collection statistics for the native and derivative Hb-C data sets used in phase calculation*

	Native	Hg^{2+}	Au^{3+}	Pt^{4+}	Pt^{4+}
No. of reflections	4673	1684	1612	588	1411
Resolution (Å)	2.4	3.5	3.5	5.0	3.5
% Completeness	82.5	77.5	74.1	79.6	64.9
MFID (%)*		22.1	10.0	16.7	8.2

* MFID stands for mean fractional isomorphous difference defined as $\sum(||F_{pH}| - |F_p||)/\sum(|F_p|)$.

Table 2. *Final heavy-atom positions for each of the derivatives used in phase calculation*

Derivative	Site number	X	Y	Z	Occupancy
Hg^{2+}					
	1	0.726	0.000	0.658	0.732
	2	0.414	−0.036	0.795	0.114
Au^{3+}					
	1	0.402	0.605	0.628	0.242
	2	0.250	0.446	0.840	0.092
Pt^{4+}					
	1	0.590	0.096	0.379	0.315
	2	0.225	0.222	0.394	0.358
	3	0.776	0.425	0.180	0.163
Pt^{4+}					
	1	0.602	0.091	0.372	0.196

reported amino-acid sequence (McDonald *et al.*, 1992). There is an additional glycine residue at the N-terminus (acetyl-GG) and no asparagine (-N) at the C-terminus. The *R* value of the final model using the 5.0 to 2.5 Å resolution shell of data is 0.16 and the deviations from ideal geometry (*X-PLOR*) are as follows: bond lengths 0.014 Å, bond angles 3.6°, dihedral angles 22.1°, and improper angles 1.4°. Fig. 1 is a ribbon drawing of the Hb-C hemichrome structure showing the 'myoglobin fold' (helices *A* to *H*, heme group between the *E* and *F* helices), hemichrome coordination where the distal histidine occupies the sixth coordination site of the Fe atom, extended termini and four internal solvent molecules.

In general the eight helices of the Hb-C structure form regular α-helices with the standard $n+4$ hydrogen bonding. The *A* helix begins with Thr13, which is a highly conserved residue at that position, and continues through Met28. Ser33 to Tyr45 make up the *B* helix, followed quickly by Asp46 to Lys52 in the *C* helix. After the *CD*-loop region the *D* helix begins with Ser60 and continues to Ser66. The *D* helix is not present in vertebrate α chains or some invertebrate hemoglobin structures, notably the dimeric *Scapharca* hemoglobin structure. The *E* helix, including the distal histidine at 73, runs from Ser67 to Glu86. The *F* helix begins with Asp91, includes the proximal histidine at 104, ends at Leu106, and is somewhat distorted due to the presence of Pro94. The *G* helix runs from Gly110 to Glu128. Finally, the *H* helix begins with Asn134 in a turn 3,10 helix and runs to Gly150. The distal side of the heme group is surrounded by large hydrophobic residues (Fig. 2) from the *B* helix [Phe39 at *B*10, Ile42 (*B*13), Phe43 (*B*14)], *CD* region (Phe53 at *CD*1), *E* helix [Ile80 at *E*13, Met81 (*E*14)], *F* helix [Leu100 at *F*4, Val109(*FG*3)] and *G* helix [Tyr114 at *G*4, Phe117 (*G*7)]. Phe53 at *CD*1 is a heme-pocket residue and is highly conserved in both invertebrate and vertebrate hemoglobins.

Three of the four internal solvent molecules are located in the heme pocket surrounded by the hydrophobic residues typically found in this region (Fig. 1). One solvent molecule is found underneath Phe53 in the *CD* region, a second is found near the end of the *E* helix near the *G* helix surrounded by a large hydrophobic pocket, and the third is found near His73. The final internal solvent molecule is found in the *AB* turn region. None of the four internal solvent molecules reported here correspond to the internal solvent molecules observed in the *Urechis caupo* hemoglobin structure.

This is the first three-dimensional structural report of hemichrome Fe-atom coordination. To confirm the hemichrome coordination of the distal histidine, the proximal and distal histidines were converted to alanines and the porphyrin ring removed entirely from the coordinate list. The resulting model was subjected to a brief round of energy minimization and an *OMIT* map using amplitudes $(F_o - F_c)$ computed which clearly shows density for the missing heme group and histidyl side chains (Fig. 3). The heme Fe atom lies 0.03 Å outside the plane of the four N atoms of the porphyrin ring on the proximal side of the heme group and 0.07 Å outside all planar atoms of the porphyrin ring as measured by *X-RAY*76 (Stewart, 1976). The average Fe atom to pyrrole N distance is 2.07 Å. These numbers compare well with the distances reported for the human carbonmonoxy hemoglobin structure (Baldwin, 1980) and sperm whale carbonmonoxy myoglobin (Kuriyan, Wilz, Karplus & Petsko, 1986) where the Fe atom has six ligands and is in a low-spin state. In contrast, the deoxy forms of myoglobin and hemoglobin have Fe atom to mean N plane distances of 0.4 to 0.6 Å (Nobbs, Watson & Kendrew, 1966; Fermi, 1975; Perutz, Hasnain, Duke, Sessler & Hahn, 1982). Distance and angle measurements on the Hb-C model were made using *FRODO* (Jones, 1979). The ε-amino groups of both the proximal and distal histidines are 2.0 Å from the heme Fe atom and the angle 73Nε—Fe—Nε104 is 176.8°. The two imidazole groups are rotated about 120° with respect to one another. The angles between the heme Fe atom and all four N atoms of the porphyrin ring are 90.0 ± 0.8°. Errors in the protein coordinates are in the neighbourhood of 0.2 Å for an X-ray model at 2.5 Å resolution.

Fig. 4 is a Ramachandran plot (Ramachandran & Sassickharan, 1968) showing the φ, ψ angles for the final Hb-C model. The outliers arise mainly from residues in the long N and C terminal tails of Hb-C. The extended tails before the *A* helix and after the *H* helix are longer than those found in many other invertebrate hemoglobin structures. Fig. 5 is a plot of the temperature factors (Å^2) for the main- and side-chain atoms as a function of

Table 3. *MIR phasing statistics for each derivative as a function of resolution where f_H is the r.m.s. heavy-atom structure factor and E the lack of closure*

Resolution (Å)	16.67	9.28	7.37	6.25	5.52	4.97	4.58	4.27	4.00	3.79
Hg^{2+}										
No. of reflections	48	110	133	154	188	199	196	213	239	220
f_H	77	73	66	62	56	53	46	46	40	39
E	63	47	30	25	26	28	29	26	24	25
Au^{3+}										
No. of reflections	48	110	133	154	188	200	197	213	239	221
f_H	27	25	23	23	20	19	19	17	16	15
E	12	11	12	11	10	9	10	10	12	12
Pt^{4+}	48	110	133	154	188	9				
f_H	52	46	43	40	37	31				
E	19	17	14	13	14	12				
Pt^{4+}										
No. of reflections						188	197	213	239	220
f_H						14	13	16	15	15
E						11	14	12	11	11
FOM	0.871	0.856	0.802	0.787	0.741	0.683	0.629	0.608	0.582	0.575

residue number. This figure shows high values for the first and last ten residues of the model. Average temperature factors for the specific groups are: all protein atoms 37.4 Å^2, main-chain atoms 41.2 Å^2, side-chain atoms 35.0 Å^2, heme-group atoms 35.8 Å^2, and solvent molecules (O atoms) 48.6 Å^2.

Solvent molecules were added using NED and difference Fourier peaks that also allowed for hydrogen-bond formation. Solvent molecules that had large shifts in *X*, *Y*, *Z* positions after energy minimization or poor density were removed. A total of 111 solvent molecules are in the final model for Hb-C. In general, the final solvent molecules form a layer around the outer surface of the protein. Four appear to be internal solvent molecules, 23 interact with main-chain atoms, 38 bond with side-chain atoms, and 46 solvent molecules interact with other solvent molecules. Three of the internal solvent molecules are in the heme region.

Discussion

The structure of a hemichrome monomeric hemoglobin, Hb-C, from *Caudina arenicola* has been determined by

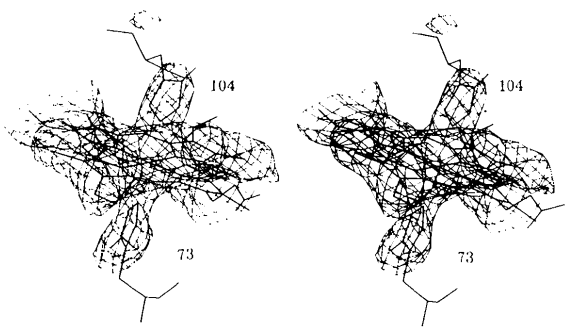

Fig. 3 $(F_o - F_c)\alpha_c$ difference electron-density map of the heme region after the heme and two histidine side chains were removed for the *OMIT* map. Electron density is contoured at 1.5σ.

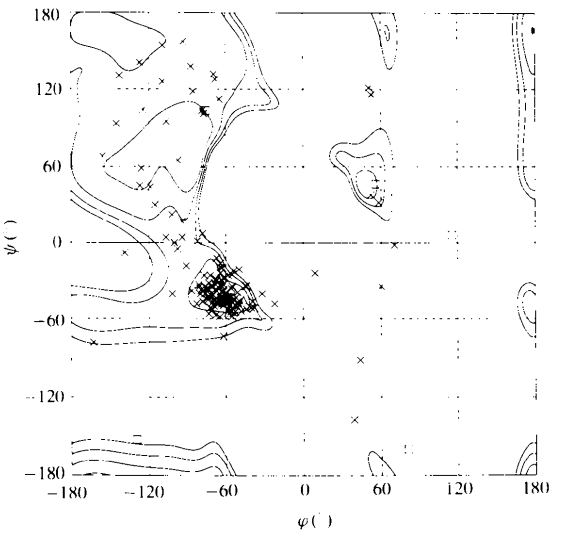

Fig. 4. Ramachandran plot of the Hb-C model (triangles are Pro, squares are Gly). The outliers are residues in the N- and C-terminal regions.

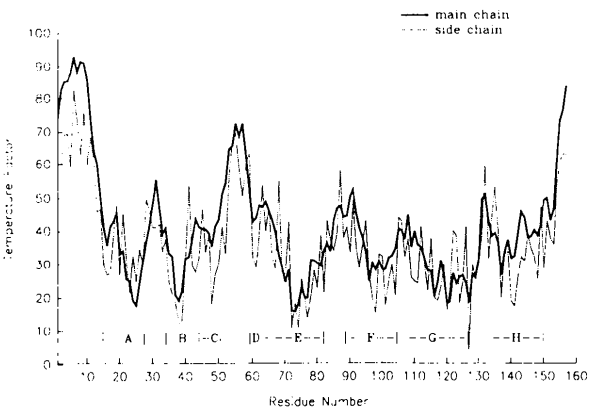

Fig. 5. Plot of temperature factors (Å^2) *versus* residue number for both main-chain and side-chain atoms. Values for the helical regions (labelled) are lower than those for the N and C terminal regions.

X-ray methods at a resolution of 2.5 Å. The secondary and tertiary structures resemble other hemoglobin tertiary structures with eight α-helices forming a heme pocket between the *E* and *F* helices. The unique aspects of this structure are the hemichrome coordination of the Fe atom of the porphyrin ring and the long N- and C-terminal tails.

The hemichrome coordination arises from the met form after Hb-C has been exposed to oxygen for 2 to 3 h. Fig. 3 shows difference electron density illustrating the coordination of both the proximal and distal histidines to the Fe atom. The Fe atom lies within 0.03 ± 0.2 Å of the plane of the porphyrin ring. The additional iron–histidine bond may cause changes in the teritary structure of Hb C that explain why the molecule, a dimer in the oxy and cyanomet states, dissociates into monomers when it forms a hemichrome. The three-dimensional structure of the cyanomet (dimeric) Hb-D molecule from *Caudina arenicola* (currently in progress) may reveal differences in the *E* and *F* helical regions of the Hb-C and D chains that could account for the difference in oligomeric states.

Another interesting aspect of hemoglobins is the evolution of hemoglobin genes and resulting amino-acid sequences. The wide diversity of organisms with hemoglobins or hemoglobin-like genes suggests the presence of an ancestral globin gene prior to the divergence of prokaryotes and eukaryotes. Recent review articles on the origin, evolution and organization of invertebrate hemoglobin genes as well as amino-acid sequence comparisons of invertebrate hemoglobins are given in Riggs (1991), Vinogradov, Walz & Pohajdak (1992) and Vinogradov *et al.* (1993).

The N- and C-terminal tails of the *Caudina arenicola* hemoglobins may provide clues to its evolutionary relationship to other invertebrate hemoglobins. The long N- and C-terminal tails of Hb-C are unusual for invertebrate as well as vertebrate hemoglobins. The structure of the dimeric *Scapharca inaequivalvis* hemoglobin has a similar N-terminal tail, although wound into a regular α-helix, but is lacking the C-terminal tail. The lack of any organized secondary structure and large temperature factors for the residues in these regions suggest a high degree of mobility. In vertebrate hemoglobins, molecules like 2,3 biphosphoglycerate (BPG) and inositol hexophosphate are known to modify the effects of ligand binding. Experiments with the *Caudina arenicola* coelomic hemoglobins have shown that ATP and organic phosphates have no effect on ligand affinity (Bonaventura *et al.*, 1976). Both hemoglobins C and D from *Caudina* are acetylated at the N terminus, and many other sea cucumber hemoglobins are also blocked at the N terminus (Kitto, Erwin, West & Omnaas, 1976). N-terminal acetylation has also been found in a number of vertebrate hemoglobins (Kitto *et al.*, 1976). In addition, a new intron in the N-terminal (NA) region of hemoglobins C (and D) between Gly10 and Asp11 has been found (Thomas, 1994). However, amino-acid sequence comparisons of the NA regions show little similarity between hemoglobins from different organisms (Thomas, 1994). This may mean that the long N- and C-terminal tails limit the effects of modifying agents and/or alter the nature of the quaternary structure.

The three-dimensional structure of Hb-C is the first example of an invertebrate hemoglobin structure from the Echinoderm phylum closely related to the vertebrates. Further studies on these invertebrate hemoglobins may help us understand the evolution of the many characteristic properties found in human hemoglobins. Towards that end, the Hb-C structure is serving as a search model for a molecular-replacement solution for the cyanomet-liganded Hb-D structure from the same organism. Determination of the Hb-D structure would allow modelling experiments of the heterodimers found in the blood cells of the sea cucumber and comparisons with other dimeric invertebrate hemoglobin structures.*

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* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1HLB, R1HLBSF). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: GR0383).

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