servation that exchange of the slowest protons in lysozyme occurs from states that thermodynamically resemble the activated complex for unfolding more closely than the fully unfolded state itself (Gregory et al., 1982).

The alternative analysis of the BPTI data presented here clearly demonstrates the importance of the water-catalyzed reaction. The contribution of water catalysis to the hydrogen exchange kinetics of other proteins remains to be elucidated. However, there is little reason to doubt that its contribution to protein hydrogen exchange is quite general.

Registry No. Poly(DL-alanine), 25281-63-4; poly(DL-alanine), SRU, 26283-00-1; BPTI, 9087-70-1; Tyr, 60-18-4; Gln, 56-85-9; Arg, 74-79-3; Ile, 73-32-5; Gly, 56-40-6; Phe, 63-91-2; hydrogen, 1333-74-0; deuterium, 7782-39-0; tritium, 10028-17-8; water, 7732-18-5.

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Dimeric Hemoglobin of the Bivalve Mollusc *Anadara broughtonii*: Complete Amino Acid Sequence of the Globin Chain†

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ABSTRACT: The complete amino acid sequence of a dimeric hemoglobin (HbI) from the marine bivalve mollusc *Anadara broughtonii* was determined by sequencing of the intact chain and peptide fragments produced by cleavage at two asparaginylglycine bonds and at methionyl, arginyl, and tryptophanyl residues. The clam hemoglobin consists of two identical polypeptide chains. The globin chain has 146 amino acid residues with a proline at the NH_2 terminus and a leucine at the COOH terminus. The calculated molecular mass of the native hemoglobin was 32945 daltons. The clam hemoglobin contains only two histidine residues, which correspond to the distal and proximal heme-linked positions. Compared with human β chain, an additional segment of seven residues is present in the NH_2-terminal region and also five less residues in the COOH-terminal region. Although such an amino-terminal elongation has been known to be characteristic of hemoglobins from the most primitive living vertebrates *Cyclostomata*, a very similar structure was found to occur in the hemoglobin from the primitive invertebrate arcid clam.

Invertebrate respiratory pigments having roles as oxygen carriers include a variety of metal–proteins (e.g., hemoglobin, hemocyanin, hemerythrin, and chlorocruorin).

Clams of the primitive family Arcidae (the so-called blood clam) have intracellular hemoglobin in the hemolymph. Hemoglobins of a few species of Arcidae (*Anadara satowi*, *Anadara broughtonii*, *Anadara senilis*, and *Scapharca inaequivalvis*) consist of two components, i.e., dimeric HbI and tetrameric HbII.[1] Both components bind oxygen cooperatively, but with no alkaline Bohr effect (Ohnoki et al., 1973; Furuta et al., 1977; Djangmah et al., 1978; Chiancone et al., 1981).

We have examined the correlation between structure and function of *A. broughtonii* hemoglobins. The major findings are as follows: (1) HbII on oxy-liganded form has an $\alpha_2\beta_2$ structure which is rare among other invertebrates. On deoxygenated form HbII shows polymerization to a highly aggregated state. (2) HbI has two like chains, which are different from either of α and β chains (Furuta et al., 1977, 1980, 1981).

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[1] Abbreviations: Hb, hemoglobin; TosPheCH$_2$Cl, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; Quadrol, *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylenediamine; DMAA, dimethylallylamine; Tris, tris(hydroxymethyl)aminomethane; NaDodSO$_4$, sodium dodecyl sulfate.

0006-2960/83/0422-0917$01.50/0

Recently, very similar features were also reported for another arcid clam, *S. inaequivalvis* (Chiancone et al., 1981).

In order to characterize these heme–heme interactions at the molecular level, it is necessary to determine the chemical structures of the components. Here we present the amino acid sequence of the globin chain of the dimeric HbI from *A. broughtonii*. This sequence is the first known, complete one of a hemoglobin from the family Arcidae. It was shown that the quaternary structure of HbI can be described as a homo dimer constructed of two identical polypeptide chains. Sequence homology with hemoglobins from many other animals is approximately 20% at maximum. The structural properties, heme contacts, subunit interfaces, and other functional amino acid residues of the *A. broughtonii* dimeric hemoglobin are discussed.

Experimental Procedures

Materials. The dimeric hemoglobin (HbI) of *A. broughtonii* and its globin were isolated and purified essentially as previously described (Ohnoki et al., 1973; Furuta et al., 1977). Most of the globin was reduced and S-carboxymethylated with iodoacetic acid by the method of Crestfield et al. (1963).

Trypsin (treated with TosPheCH$_2$Cl) was obtained from Worthington Biochemical Corp. and carboxypeptidase Y from Oriental Yeast Co.

Iodoacetic acid, cyanogen bromide, and all other sequencing reagents were purchased from Wako Pure Chemical Co. All other materials were of the highest grade commercially available. Iodoacetic acid was recrystallized from chloroform before use. Thin-layer plates used were silica gel 60 F_{254} (Merck) or Wakogel FM (Wako Pure Chemical Co.).

Cleavage at the Methionines. Lyophilized, salt-free S-carboxymethylated protein (4.5 μmol) was dissolved in 70% formic acid (0.9 μmol/mL). After addition of a 100-fold excess of cyanogen bromide over the methionine content, the mixture was incubated at 27 °C in the dark for 20 h. The reaction mixture was then diluted 10-fold with cold water and lyophilized twice from the same volume of cold water.

Cleavage at the Tryptophans. A part of globin (4.2 μmol) was oxidized by performic acid, instead of the carboxymethylation described above. The oxidized globin was succinylated with 2-fold (w/w) excess of succinic anhydride in 0.1 M NaCl for 1 h. The pH of the mixture was maintained at 9.0 by the addition of 2 M NaOH. The protein was desalted by using a Sephadex G-25 column (2.5 × 34 cm) equilibrated with 0.02 M ammonium bicarbonate. The protein fraction was collected and lyophilized.

The oxidized and succinylated protein was digested according to the method of Ozols & Gerald (1977). To the protein (2 μmol) was added 1 mL each of 88% formic acid and anhydrous heptafluoro-*n*-butyric acid. After addition of 60-fold (w/w) of solid cyanogen bromide to the above mixture, the solution was incubated at room temperature in the dark for 24 h. The mixture was next dried with a stream of nitrogen gas. The hydrolysate was suspended with a 10-fold volume of cold water and lyophilized.

Cleavage with Hydroxylamine. The cleavage of the two asparaginylglycine bonds was accomplished by the method of Bornstein (1970). The S-carboxymethylated globin chain (7.3 μmol) was incubated for 2.5 h with 40 mL of 1 M hydroxylamine in 0.1 M K_2CO_3 at 45 °C (pH 10.0). The sample was desalted by use of a Sephadex G-25 column (2.5 × 42 cm) equilibrated with 10 mM ammonium bicarbonate and then lyophilized.

Subdigestion of Fragment CB3. A carboxyl-terminal fragment CB3 (residues 75–146) obtained from cyanogen bromide digestion contained one arginine residue. Prior to cleavage, the fragment was succinylated as above, since the separation of two peptides generated was difficult. The modified fragment (12 mg) was dissolved in 2 mL of 0.1 M ammonium bicarbonate buffer (pH 9.0) and incubated with trypsin treated with TosPheCH$_2$Cl (enzyme/substrate molar ratio of 1/50) for 3 h.

Designation of Fragments. Fragments generated by each method of cleavage are numbered consecutively, from the amino-terminal sequence to the carboxyl-terminal end of the globin molecule. Cyanogen bromide fragments are designated numerically as CB1–CB3. Fragments obtained by cleavage with hydroxylamine are designated by the symbol Hy, fragments digested at tryptophan residues are given the symbol Tp, and tryptic peptides from fragment CB3 are labeled as CB3-T.

Amino Acid Analysis. S-Carboxymethylated globin and selected fragments were hydrolyzed in vacuum-sealed tubes containing constant boiling HCl for 24 h at 110 °C. All analyses were performed on a Hitachi KLA-5 amino acid analyzer.

Sequencing Procedures. The whole protein and selected fragments were sequenced in a JEOL JAS-47K spinning cup sequencer according to the method of Edman & Begg (1967) as modified by Hermodson et al. (1972, 1977). The amino terminal sequence of the S-carboxymethylated globin and three large fragments (Tp2, CB3, and Hy2) were determined by using a standard fast protein Quadrol double-cleavage program. For the degradation of other peptides a program using DMAA as buffer was employed.

The amino acid thiazolinones were converted to phenylthiohydantoin amino acids (Guyer & Todd, 1975). Phenylthiohydantoin amino acids were identified by thin-layer chromatography and by amino acid analysis after back-hydrolysis to the original amino acid. Regeneration of the corresponding amino acid was performed by hydrolysis at 150 °C for 4 h in vacuo in constant boiling HCl containing 0.1% stannous chloride (Mendez & Lai, 1975). All amide assignments were made by thin-layer chromatography of the amino acid phenylthiohydantoins. Thin-layer chromatography on silica gel plates was carried out in *n*-heptane/1,2-dichloroethane/propionic acid (58:25:17 v/v).

Partial sequence of the carboxyl-terminal region of peptide CB2 was determined by carboxypeptidase action. Prior to enzyme digestion, the peptide CB2 was acetylated with a 50-fold molar excess of acetic anhydride for 30 min at room temperature. The pH was maintained at 8.5 by the addition of 3 M NaOH, and the sample was then lyophilized. The acylated CB2 was dissolved in 0.1 M pyridine/acetate buffer, pH 6.0, at a concentration of 1 μmol/mL, and then incubated at 27 °C with carboxypeptidase Y (molar ratio of enzyme to substrate, 1/100). Aliquots (0.2 mL) of the sample were taken at intervals and diluted with analyzer sample buffer (pH 2.2 citrate buffer). After removal of any resulting precipitates by centrifugation, the amino acids liberated were determined with the aid of the analyzer. The acetylation was effectual for excluding the overlaps of small peptides in amino acid analyses.

Results

Amino Acid Composition. The amino acid composition of dimeric hemoglobin from *A. broughtonii* was reported previously (Furuta et al., 1977). Table I shows the comparison with that derived from the complete amino acid sequence. Both data are compatible with each other except for a few small discrepancies.

Table I: Amino Acid Composition of Globin from *A. broughtonii* HbI

amino acid	acid analysis[a]	sequence
Lys	14.8	15
His	1.9	2
Arg	4.1	4
Cys	1.0	1
Asp	21.4	11
Asn		10
Thr	6.9	7
Ser	7.0	7
Glu	11.2	3
Gln		7
Pro	2.0	2
Gly	10.4	11
Ala	18.0	18
Val	11.9	13
Met	2.8	3
Ile	7.9	8
Leu	13.3	13
Tyr	2.9	3
Phe	5.9	6
Trp	1.6	2

[a] From Furuta et al. (1977).

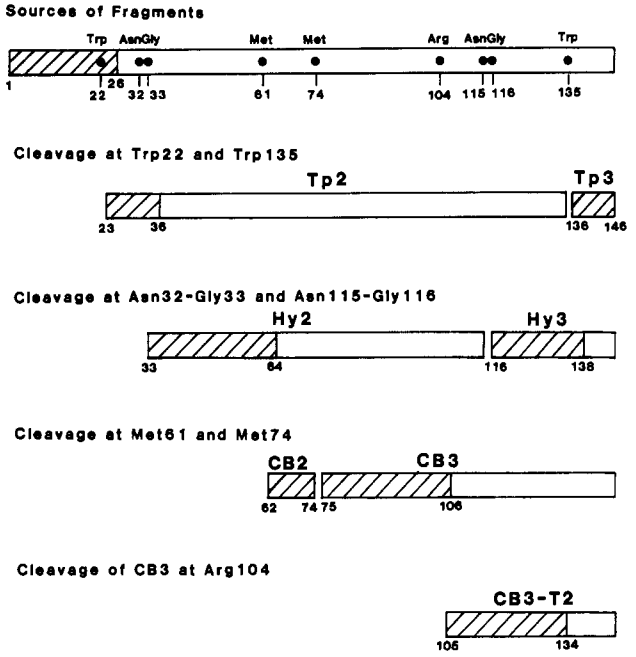

FIGURE 1: Summary of the sequencing strategy. The top bar represents the whole globin and the numbered residues that are important for its fragmentation. Crosshatched section in each bar denotes the portion determined by sequence analysis.

Sequence Analysis. The strategy used to establish the primary structure is outlined in Figure 1. Automated Edman degradations were performed on the whole globin chain and seven fragments derived from the protein. Purification of the fragments by gel filtration chromatography led to the results shown in Figure 2. Fragments CB2, CB3, Hy2, Hy3, and Tp3 were each obtained as pure peptides. Their amino acid compositions are shown in Table II.

The sequence of the largest fragment Tp2 (residues 23–135) was determined on an aliquot of peak I peptide (see Figure 2b). Sequencer analysis of fragment CB3-T2 (105–146) was carried out on a tryptic digest of succinylated CB3. The first step of degradation on two aliquots has yielded only Pth-lysine.

The amino acid composition of the globin shows three methionine residues are present (Table I). However, the number of major fragments generated with cyanogen bromide was three and not four. Two homoserine residues were calculated to be present from amino acid analysis data of peptide CB1, indicating the presence of an internal homoserine residue. Sequence studies of fragment Hy2 revealed the presence of a Met–Thr bond at position 37–38. Such a resistant bond to cyanogen bromide cleavage has been reported by Schroeder et al. (1969).

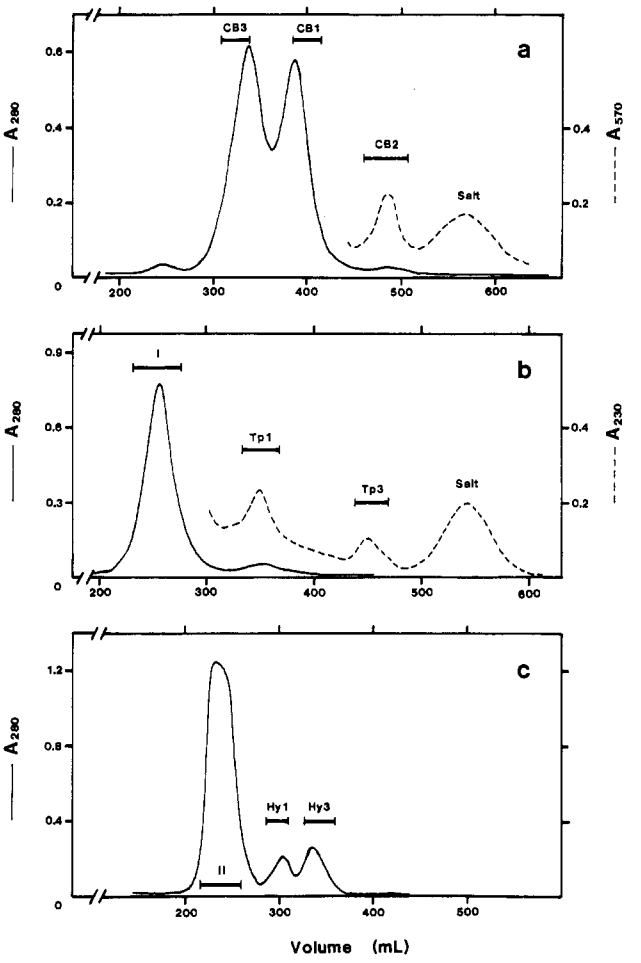

FIGURE 2: (a) Gel filtration of the fragments generated by cleavage of S-carboxymethylated globin at methionyl residues. The digests (4.3 μmol) were applied to a column (2.5 × 120 cm) of Sephadex G-75 in 2% formic acid. The flow rate was 10 mL/h. A part of the effluent fractions was monitored by absorbance at 570 nm (---) after ninhydrin reaction. (b) Separation of the fragments generated by cleavage of oxidized and succinylated globin at tryptophanyl residues. The digests (2.2 μmol) were applied to a column (2.5 × 115 cm) of Sephadex G-50 in 0.02 M ammonium bicarbonate (pH 8.5). The flow rate was 13 mL/h. (c) Fractionation of the fragments generated by cleavage of S-carboxymethylated globin at two asparaginylglycine bonds. The digests (4.7 μmol) were applied to a column (2.5 × 115 cm) of Sephadex G-50 in 0.1 M ammonium bicarbonate (pH 8.5). The flow rate was 15 mL/h.

Sequence. The complete primary structure of the hemoglobin subunit (HbI) from *A. broughtonii* is presented in Figure 3. The globin consists of 146 amino acid residues in a single polypeptide chain. The calculated molecular weight of the globin from the sequence data was 15 856. This value is in accord with the 15 500 value, obtained from NaDodSO$_4$–polyacrylamide gel electrophoresis (Furuta et al., 1977). The molecular weight of the dimeric HbI containing two protohemes was calculated as 32 945 (for additional information see paragraph at end of paper regarding supplementary material).

Discussion

The primary structure of HbI from the mollusc *A. broughtonii* is compared with those of a few vertebrates and

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                5                  10                  15
Pro Ser Val Gln Gly Ala Ala Ala Gln Leu Thr Ala Asp Val Lys

               20                  25                  30
Lys Asp Leu Arg Asp Ser Trp Lys Val Ile Gly Ser Asp Lys Lys

               35                  40                  45
Gly Asn Gly Val Ala Leu Met Thr Thr Leu Phe Ala Asp Asn Gln

               50                  55                  60
Glu Thr Ile Gly Tyr Phe Lys Arg Leu Gly Asn Val Ser Gln Gly

               65                  70                  75
Met Ala Asn Asp Lys Leu Arg Gly His Ser Ile Thr Leu Met Tyr

               80                  85                  90
Ala Leu Gln Asn Phe Ile Asp Gln Leu Asp Asn Thr Asp Asp Leu

               95                 100                 105
Val Cys Val Val Glu Lys Phe Ala Val Asn His Ile Thr Arg Lys

              110                 115                 120
Ile Ser Ala Ala Glu Phe Gly Lys Ile Asn Gly Pro Ile Lys Lys

              125                 130                 135
Val Leu Ala Ser Lys Asn Phe Gly Asp Lys Tyr Ala Asn Ala Trp

              140                 145
Ala Lys Leu Val Ala Val Val Gln Ala Ala Leu
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FIGURE 3: Complete amino acid sequence of *A. broughtonii* HbI.

invertebrates in Figure 4. The sequence homology of the arcid clam globin with other globins is as follows: with human β chain, 21.9%; with lamprey *Petromyzon*, 19.9%; and with mollusc *Aplysia*, 22.6%. Homology with human α chain (Braunitzer et al., 1961; Konigsberg et al., 1961), *Glycera* (Imamura et al., 1972), *Chironomus* CTT-IIβ (Kleinschmidt & Braunitzer, 1976), and soybean leghemoglobin (Ellfolk & Sievers, 1971) is rather small, 14–18%.

Crystal structure analysis have provided structures for several hemoglobins and myoglobins from mammals (e.g., Ladner et al., 1977), a sea lamprey (Hendrickson et al., 1973), an annelid worm *Glycera* (Padlan & Love, 1974), a larval insect *Chironomus* (Steigemann & Weber, 1979), and a le-

Table II: Amino Acid Composition of the Selected Fragments[a]

fragment	CB2	CB3	Hy2	Hy3	Tp3
position in sequence	62–74	75–146	33–115	116–146	136–146
yield (%)	73	62	35	39	21
N-terminal residue	Ala	Tyr	Gly	Gly	Ala
amino acid					
Lys	0.9 (1)	8.2 (8)	5.4 (5)	4.5 (5)	0.9 (1)
His	0.8 (1)	1.2 (1)	1.5 (2)		
Arg	0.9 (1)	1.4 (1)	3.0 (3)		
Cys		0.8[b] (1)	0.7[b] (1)		
Asp	2.1 (2)	11.3 (11)	13.0 (13)	3.0 (3)	
Thr	1.2 (1)	2.3 (2)	5.5 (6)		
Ser	0.9 (1)	2.3 (2)	3.4 (3)	1.1 (1)	
Glu		5.1 (5)	7.0 (7)	1.2 (1)	1.1 (1)
Pro		0.8 (1)		1.2 (1)	
Gly	1.2 (1)	3.4 (3)	6.2 (6)	2.1 (2)	
Ala	1.0 (1)	10.6 (11)	7.4 (7)	6.9 (7)	4.0 (4)
Val		7.1 (8)	5.6 (6)	3.4 (4)	2.7 (3)
Met	1.0[c] (1)		2.7 (3)		
Ile	0.9 (1)	4.6 (5)	5.9 (6)	0.9 (1)	
Leu	1.9 (2)	5.7 (6)	7.8 (8)	2.9 (3)	1.8 (2)
Tyr		1.7 (2)	1.8 (2)	0.8 (1)	
Phe		3.6 (4)	4.6 (5)	0.9 (1)	
Trp[d]	ND	ND (1)	ND	ND (1)	ND

[a] Values in parentheses are compositions derived from sequence analysis. [b] Detected as *S*-(carboxymethyl)cysteine. [c] Detected as homoserine and homoserine lactone. [d] ND, not determined by amino acid analysis.

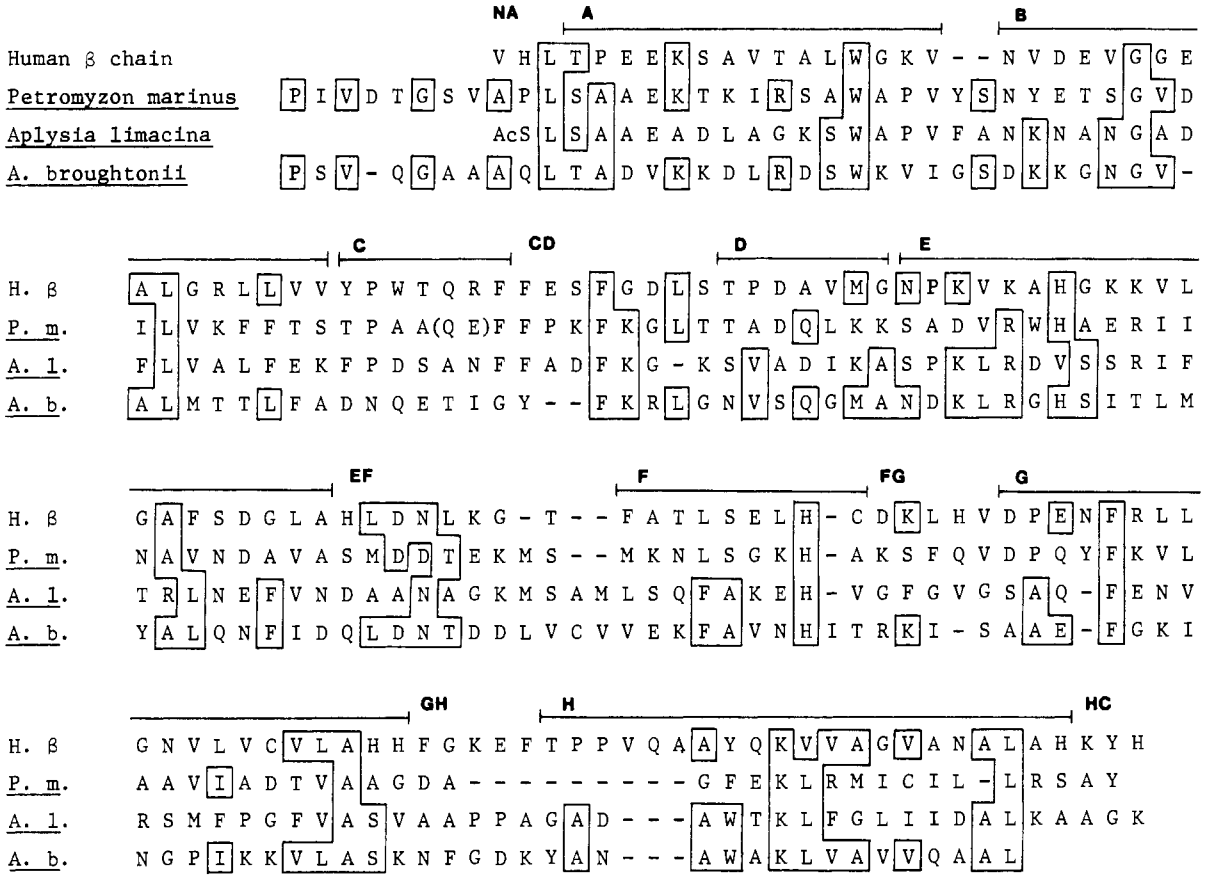

FIGURE 4: Comparison of the amino acid sequence of globin from *A. broughtonii* HbI with those of from β chain of human HbA (Braunitzer et al., 1961), sea lamprey *Petromyzon marinus* hemoglobin (Li & Riggs, 1970), and mollusc *Aplysia limacina* myoglobin (Tentori et al., 1973). The chains are aligned on a basis of the compilation of Goodman (1976, 1981) and in addition of our slight modification. Identical residues from other species are enclosed in boxes. Gaps (—) are introduced to achieve maximum homology, and standard single-letter abbreviations are used. Ac indicates the protein is N-acetylated. The helix designations are from Perutz et al. (1968).

ghemoglobin from the root nodules of the yellow lupin (Vainshtein et al., 1977). These globins from many different organisms have very different primary structures but remarkably similar secondary and tertiary structures. Lesk & Chothia (1980) discuss how proteins with different amino acid sequences form very similar protein structures. The fold of the globin chain from the clam *A. broughtonii* may also be retained. The only histidines in the clam hemoglobin are two that are homologous to the distal and proximal heme-linked positions.

Reversible oxygen binding is the most fundamental hemoglobin function. The amino acid residues at heme contacts are very homologous. Sequence homology in the heme contact region of the clam with horse α and horse β chains reaches up to about 70%. The environment of the heme prosthetic group in this arcid clam hemoglobin also appears to be a well-preserved region. In mammalian hemoglobin or myoglobin, on the distal side of the heme plane the oxygen molecule lies in a substantial pocket, bound by two hydrophobic groups (Phe-CD1 and Val-E11) and the imidazole group of the distal His-E7 (Phillips & Schoenborn, 1981). In clam HbI, the residues at positions CD1 and E11 are replaced by tyrosine and leucine, respectively. These distinctive substitutions by somewhat bulky but similar groups are likely to influence the ligand binding by hemoglobin. In fact, the *A. broughtonii* HbI shows relatively low oxygen affinity, P_{50} = 12.7 torr (at 25 °C; Furuta et al., 1980).

One of the most remarkable features in the structure of this clam globin is the elongation by seven residues at the amino-terminal region (Figure 4). This peptide elongation and also the presence of proline at the NH_2 terminus are common features in the hemoglobins of the most primitive living vertebrates such as *Cyclostomata*, which include lampreys, *Petromyzon marinus* and *Lampetra fluviatilis* (Li & Riggs, 1970; Zelenik et al., 1979), and the hagfishes, *Eptatretus stoutii* and *Myxine glutinosa* (Li & Riggs, 1972; Liljeqvist et al., 1979). The fact that these hemoglobins have very similar structures in the amino-terminal region may have molecular evolutionary implications.

In contrast, the carboxyl-terminal region of the clam globin lacks five residues when compared with the human β chain (Figure 4). Such a carboxyl terminal shortening and the lack of an HC segment are rare among the hemoglobins and myoglobins so far sequenced. However, the carboxyl-terminal sequence of the clam globin is very homologous with the relevant H-helix region of the mammalian β chain. Seven of 13 residues (H7–H19) in the C-terminal region are identical. The C-terminal amino acids of clam globin is Leu-146 (H19), which is invariant and in contact with heme prosthetic group in mammals. Therefore, the carboxyl-terminal leucine in clam hemoglobin also may be fixed in a rigid state.

In the previous communication, we demonstrated that *A. broughtonii* hemoglobins neither show an alkaline Bohr effect nor are effectively organic phosphates. The absence of Bohr proton ionizing groups could account for the absence of the Bohr effect (Furuta et al., 1977, 1980). Several functional groups are known to be responsible for the heterotropic interactions in mammalian hemoglobins. Amino acids which affect the Bohr proton ionization include α1-Val (NA1), α122-His (H5), β1-Val (NA1), β2-His (NA2), β143-His (H21), and β146-His (HC3) (Perutz et al., 1980) and α20-His (B1) and α89-His (FG1) (Ohe & Kajita, 1980). The binding site of 2,3-diphosphoglyceric acid is also constituted by β1 Val (NA1), β2-His (NA2), β82 Lys (EF6), and β143 His (H21) (Arnone, 1972). Since none of the corresponding residues occur in the clam globin sequence (cf. Figure 4), it is clear that the clam dimeric HbI, unlike mammalian hemoglobins, lacks the allosteric regulation mechanism by proton or organic phosphates.

We have also reported that the modification of HbI with *p*-chloromercuribenzoate induced a very increased oxygen affinity (ca. 85-fold, compared to native HbI; Furuta et al., 1980). The modified SH groups can be assigned as Cys-92 at the EF corner, because there is only one cysteine residue in the globin chain.

Therefore, the great increase of ligand affinity may be also attributed to a configurational change of the distal His–heme–proximal His linkage, induced by introduction of a bulky group to the bending portion of a "hairpin" like structure between E and F helices.

The oxygen association–dissociation equilibrium of the *A. broughtonii* HbI shows high cooperativity (n = 1.8 in 0.05 M Tris-HCl buffer, pH 7.4; Furuta et al., 1980). Although the unlike chain interaction between α and β is very important for the structural changes related to ligand binding in mammalian hemoglobins, *A. broughtonii* HbI is a homo dimer consisting of a single kind of chain. A comparison of the contact regions between the subunits of dimeric HbI and those of mammalian hemoglobins shows no conservation of the amino acids concerned. The homology of the clam HbI with horse hemoglobin (Ladner et al., 1977) were 14.3% in the residues of $\alpha_1\beta_1$ contacts and 5.6% in those of $\alpha_1\beta_2$ contacts, even if structurally similar amino acids were added. Cooperative dimeric hemoglobins have also been found in other species of the arcid clams (Ohnoki et al., 1973; Djangmah et al., 1978; Chiancone et al., 1981), in the holothurian echinoderms (Manwell, 1966; Terwilliger & Read, 1972), and in the polychaete annelid *Glycera* (Vinogradov et al., 1970). The gastropods mollusca *Busycon canaliculatum*, *Buccinum undatum*, and *Nassa mutabilis* have cooperative dimeric myoglobins in the radular muscle (Terwilliger & Read, 1971; Geraci et al., 1977). The amino acid sequence of the *Busycon* myoglobin was determined (Bonner & Laursen, 1977). The maximum homology of *A. broughtonii* HbI with this dimeric myoglobin was 21.2%.

The body fluid of the insect larvae of *Chironomus thummi thummi* contains as many as 11 components which are monomeric or dimeric hemoglobins. From the analyses of these primary structures, five components have been demonstrated to be homo dimer (Kleinschmidt & Braunitzer, 1976, 1980; Steer & Braunitzer, 1981; Aschauer et al., 1981; Aschauer & Braunitzer, 1981).

Thus, several invertebrates possess the respiratory pigments having dimeric subunit structure, and some of them show special homotropic interactions different from vertebrate hemoglobins. It is interesting to note that lamprey hemoglobins show cooperativity between liganded monomer and deoxygenated polymer (i.e., homo polymer) (Briehl, 1963; Dohi et al., 1973).

Recently, preliminary crystallographic data on monomeric and dimeric hemoglobins from sea cucumber *Molpadia arenicola* were reported by Carson et al. (1979). Their detailed structures might shed light in understanding the molecular mechanism of dimeric cooperativity and the evolutionary relationships between vertebrate and invertebrate hemoglobins.

Acknowledgments

We are grateful to Professor Tadashige Habe of the University of Tokai, the School of Marine Science and Technology, for the identification of the clam and to Drs. Shin-ichi Nagai and Kiyoshi Ishii, the Department of Biology,

for useful advice. We also thank Yuriko Nozawa, Kimiko Ushiku, and Eiko Sakamoto for technical assistance.

Supplementary Material Available

Tables III–X and Figures 5 and 6 giving (a) the details of automated sequence analysis of S-carboxymethylated globin and generated fragments, including repetitive yields and the yield in nanomoles at each step, (b) the results of hydrolysis of fragment CB2 by carboxypeptidase Y, (c) the isolation of fragment Hy2, (d) amino acid analyses of N-terminal fragments CB1, Hy1, and Tp1, and (e) comparison of *A. broughtonii* HbI with horse hemoglobin at heme contact and subunit interfaces (11 pages). Ordering information is given on any current masthead page.

Registry No. *A. broughtonii* HbI, 84130-19-8.

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