During adaptation to low temperatures, Antarctic fishes have acquired special hematological features which clearly differentiate them from fishes of temperate and tropical climates. The hematocrit and hemoglobin (Hb) concentration are highly different from those shown by blood, intact erythrocytes, and unstripped hemolysates. Both hemoglobins have unusually high oxygen affinity and display a relatively small Bohr effect; the Root effect is elicited only by organophosphates and is also reduced. Remarkably, the Hill coefficient is close to one in the whole pH range, indicating absence of cooperative oxygen binding which, in A. orianae hemoglobin, could be ascribed to the subunit heterogeneity shown upon oxygen dissociation. In comparison with the other families of the suborder Notothenioidei, the oxygen-transport system of these two species of Artedidraconidae has unique characteristics, which raise interesting questions on the mode of function of a multisubunit molecule and the relationship with cold adaptation.

The hemoglobin of Antarctic fishes belonging to the family Artedidraconidae, Ar- tedidraconidae, includes six families with 120 species, 95 of which are Antarctic (5, 6): Bovichtidae, Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae, and Channichthyidae (in fact, the families might be seven, since recent evidence (7) suggests that Bovichtidae should be grouped into Pseudaphritidae and Bovichtidae). Notothenioids generally have a single major Hb (Hb 1), often accompanied by a minor component (Hb 2), accounting for approximately 95 and 5% of the total, respectively (8–10). A cathodal Hb (Hb C) is present in trace amounts, except in Trematomus newnes, of the family Nototheniidae, in which Hb C is approximately 25–30% of the total (11).

In this study, the oxygen-transport system of species of the family Artedidraconidae, which comprises 24 of the 80 red-blooded Antarctic species of the suborder Notothenioidae so far identified (5, 6), was thoroughly investigated for the first time. Artedidraconids are benthic fish, have a wide depth distribution, and are largely confined in the Antarctic continental shelf and slope (12). This paper reports the complete amino acid sequence of the subunit heterogeneity shown upon oxygen dissociation. The functional features of Hbs were very similar to those measured in whole blood, intact erythrocytes, and unstripped hemolysates.

**EXPERIMENTAL PROCEDURES**

DEAE-cellulose (DE52) was from Whatman; trypsin (EC 3.4.21.4), treated with 1-1-tosylamide-2-phenylethylchloromethyl ketone, from Cooper Biomedical; Tris and bisTris from Sigma; dithiothreitol from Fluka; all sequanal-grade reagents from Applied Biosystems; HPLC-grade acetonitrile from Lab-Scan Analytical. All other reagents were of the highest purity commercially available.

A. orianae was caught by means of Agassiz Trawl in the northeastern Weddell Sea, Antarctica, and P. scotti by gill nets in the vicinity of Terra Nova Bay Station, Ross Sea, Antarctica. Immediately after catch, fish were transferred to aquariums supplied with running, aerated seawater at approximately −1.0 °C.

Blood samples were drawn from the caudal vein of living animals by means of heparinized syringes; the red blood cells were washed three times in isotonic saline solution (1.7% NaCl, in 1 mM Tris-HCl, pH 8.1). Hemolysates were prepared as described (13). Hb concentration and purification from minor contaminants was carried out by ion-exchange chromatography on a DE52 column (1 × 3 cm), equilibrated with 10 mM Tris-HCl, pH 8.1, and eluted with 100 mM Tris-HCl, pH 7.1. Gel filtration was performed by fast protein liquid chromatography (Pharmacia) on a ProteinPak 300 SW column ( Waters), equilibrated with 50 mM Tris-HCl, pH 8.0, containing 100 mM NaCl, at a flow rate of 0.2 ml/min.

Globins were precipitated with 10 volumes of acetone containing 5 mM HCl at −20 °C; α and β chains were separated by reverse-phase HPLC on a μBondapak C18 column (Waters, 0.39 × 30 cm). In P. scotti,
the eluents were (A) 45% acetonitrile containing 0.3% trifluoroacetic acid and (B) 55% acetonitrile; a linear gradient from 0 to 100% of eluent B in 15 min was used. In A. oriana, the eluents were (A) 0.1% trifluoroacetic acid and (B) acetonitrile containing 0.08% trifluoroacetic acid; the elution was carried out with a multistep gradient of eluent B. Flow rate was 1 ml/min. Cellulose acetate electrophoresis of hemolysates and purified Hbs and SDS-polyacrylamide gel electrophoresis of the purified globins were carried out as described (14, 15). Globin denaturation, alkylation of the sulphydryl groups with 4-vinylpyridine, tryptic digestion, and cleavage of Asp-Pro bonds were carried out according to described procedures (16, 17).

Tryptic peptides were purified by reverse-phase HPLC on a µBondapak C18 column (Waters, 0.39 × 30 cm), equilibrated with eluent A (0.1% trifluoroacetic acid); peptides were eluted with a multistep gradient of eluent B (acetonitrile, containing 0.08% trifluoroacetic acid). Amino acid analyses were performed with an Applied Biosystems automatic derivatizer model 420 A, equipped with the hydrolysis option and with on-line detection of phenylthiohydantoin amino acids. Alternatively, gas-phase manual hydrolysis was carried out for 1 h at 155 °C in 6 N HCl containing 1% 2-mercaptoethanol and 1% phenol.

Amino acid sequencing was carried out with an Applied Biosystems automatic sequencer model 477A, equipped with on-line detection of phenylthiohydantoin-derivatives. Sequencing of Asp-Pro-cleaved globins was performed after treatment with o-phthalaldehyde (18), to reduce the background due to sequencing from the N terminus. Molecular mass measurements were carried out with a Hewlett-Packard mass spectrometer model 5989B, equipped with the electrospray source model 59987A.

Oxygen saturation experiments were carried out at 2 °C, as described previously (15). Oxygen-equilibrium curves were obtained by the tonometric method (19) at 2, 10, and 20 °C, in the pH range 6.5–8.0. The heat of oxygenation was calculated from the integrated van’t Hoff equation,
comparison with other notothenioids (10), the Hbs showed a modest, effector-enhanced alkaline Bohr effect (becoming virtually absent at 10 °C in A. orianae Hb, not shown) in the pH range 6.5–8.0 (Fig. 4, panels A and B); the Bohr coefficient $D\log P_{50}/DpH$ was, respectively, $-0.35$ and $-0.31$ in the absence, and $-0.51$ and $-0.44$ in the presence, of the physiological effectors. Although the overall oxygen affinity of whole blood (as well as erythrocytes and unstripped hemolysate) of A. oriana and P. scotti (Fig. 4, panels A and B, respectively) was slightly lower than that measured in Hb in the presence of chloride and phosphates, the slopes of the oxygen-equilibrium curves were very similar in the pH range 7.0–7.5, where the Bohr effect is active.

In the whole pH range, the Hill coefficient of the Hbs of both species was close to one (Fig. 4, panels C and D), regardless of the presence of chloride and organophosphates, with only slightly higher values observed in P. scotti. These results were taken as strong evidence of the absence of cooperative oxygen binding.

In order to ascertain whether the low Hill coefficient values were due to subunit dissociation, A. oriana Hb was analyzed by fast protein liquid chromatography gel filtration at pH 8.0 (see "Experimental Procedures" for details). The hemoprotein was eluted from the column at the retention time of tetrameric Hb (not shown), without any apparent dissociation. Therefore, the absence of subunit cooperativity is due to intrinsic properties of the tetrameric molecule; further work is required to establish correlations with molecular structure.

The Root effect (20, 21) often displayed by fish Hbs, which leads to incomplete saturation of Hb in air, was not found, at 2 °C, in A. oriana and P. scotti Hbs in the absence of organophosphates, but it was induced to a limited extent by ATP or inositol hexakisphosphate (Fig. 5). The Root effect of A. oriana and P. scotti blood, erythrocytes, and unstripped hemolysate was very similar to that shown by the isolated Hb (Fig. 5, panels A and B).

The regulation of the oxygen affinity by temperature was investigated in the range 2–20 °C. A very strong $\Delta H$, larger than that of mammalian Hbs (22), was measured in A. oriana Hb at pH 8.0, in the absence and presence of chloride and ATP. However, lowering the pH to 7.0 brought about a dramatic decrease of the oxygen-binding enthalpy, which became almost zero in the presence of effectors (Table II).

**Oxygen Dissociation Kinetics**—Fig. 6 shows the progress curve for oxygen dissociation of Hb of A. oriana at different pH values and at 21 °C, displaying a biphasic process characterized by two exponentials, which can be attributed to a different ligand dissociation behavior for the two subunits a and b of the tetramer, even though the kinetic heterogeneity is not as marked as in the Root effect Hb of the temperate fish Chelidonichthys kumu (23). The amplitude of the process decreased as the pH was lowered (Fig. 6), indicating that A. oriana Hb was already partially deoxygenated at atmospheric oxygen pressure and at 21 °C, before mixing with sodium dithionite. However, this effect appeared markedly temperature-dependent, since at lower, physiological temperatures, no Root effect, i.e. no partial deoxygenation, was observed upon pH lowering at atmospheric pressure (Fig. 5). Both deoxygenation rate constants displayed a pH-dependent behavior in the observed pH range (Fig. 7); the data were analyzed according to,

$$k_{\text{obs}} = k_{\text{diss}}/(1 + K_\text{d}(\text{H}^+)) + k_{\text{a}}K_\text{a}(\text{H}^+)/(1 + K_\text{a}(\text{H}^+))$$  (Eq. 2)
where \( k_{\text{obs}} \) is the observed oxygen dissociation rate constant, \( k_{\text{alk}} \) and \( k_{\text{ac}} \) are the oxygen dissociation rate constants for the unprotonated and protonated Hb, respectively, and \( K_a (=10^{pK_a}) \) is the proton binding constant to oxygenated Hb. The pH dependence described by the continuous lines indicated a single protonation event with a \( pK_a = 7.1 \pm 0.15 \), closely similar for both phases and essentially unaffected by 3 mM ATP (Fig. 7, panels A and B, and Table III). This behavior suggests that the two subunits of the tetramer, although showing different values of the oxygen dissociation rate constants (as from the biphasic progress curves in Fig. 6), are functionally regulated by the same protonation process, indicating that differences in the observed rates should be related to variations in the conformation of the distal side of the heme pocket.

**Carbon Monoxide Binding Kinetics**—Fig. 8 shows the pH dependence of the rate constant for the monophasic carbon monoxide binding. The Hemoglobins of Artedidraconid Fishes
monoxide binding process to both Hbs. The behavior, substantially unaffected by 3 mM ATP (data not shown), was investigated over a very wide pH range, spanning from pH 2.5 to 9.0. This range has been shown to be suitable in order to follow the effect of the protonation of the proximal and distal histidine on the CO binding process, since over this pH interval both events are taking place (24, 25). The pH dependence was analyzed according to Equation 2 modified only in that $k$ values of Equation 2 are substituted by $l$ since the rate constants are referring to the bimolecular carbon monoxide binding rate constants. Furthermore, it must be outlined that the $K_a$ value measured in CO binding kinetics refers to the proton binding constant to unliganded Hb. The continuous lines refer to the fitting of the experimental data from the Hbs of $A$. orianae and $P.$ scotti according to Equation 2, employing the set of parameters reported in Table IV.

**TABLE I**

Amino acid composition of the $\alpha$ (A and B) and $\beta$ (C and D) chains of $A$. orianae and $P.$ scotti, respectively.

| Amino acid | A      | B      | C      | D      |
|------------|--------|--------|--------|--------|
| Asp/Asn    | 12.6(15)| 15.2(15)| 13.8(19)| 16.2(16)|
| Glu/Gln    | 6.4(5) | 5.2(5) | 11.9(11)| 11.7(10)|
| Ser        | 10.1(13)| 14.3(14)| 8.0(10) | 10.1(11)|
| Gly        | 6.7(6) | 6.8(6) | 10.7(11)| 10.7(10)|
| His        | 8.6(5) | 6.1(6) | 7.2(5)  | 8.2(9)  |
| Arg        | 3.7(3) | 3.0(3) | 4.1(4)  | 3.0(3)  |
| Thr        | 5.4(5) | 5.3(5) | 5.0(5)  | 4.6(4)  |
| Ala        | 14.7(18)| 16.9(18)| 12.8(13)| 15.1(18)|
| Pro        | 6.7(6) | 6.4(6) | 4.0(4)  | 3.8(4)  |
| Tyr        | 3.7(3) | 3.2(3) | 5.6(5)  | 5.2(5)  |
| Val        | 9.9(11)| 10.7(12)| 10.1(11)| 7.7(9)  |
| Met        | 1.6(4) | 4.5(4) | 1.2(4)  | 3.6(3)  |
| Cys        | ND(1)  | ND(1)  | ND(2)   | 1.6(2)  |
| Ile        | 10.1(10)| 7.9(9) | 8.7(9)  | 7.8(30) |
| Leu        | 14.2(13)| 13.0(13)| 14.2(14)| 15.0(16)|
| Phe        | 6.7(6) | 6.2(6) | 5.9(7)  | 8.1(8)  |
| Lys        | 15.2(16)| 15.4(16)| 8.7(8)  | 8.1(8)  |
| Trp        | ND(2)  | ND(2)  | ND(2)   | ND(2)   |
| No. of residues | 142    | 142    | 146     | 146     |
| Molecular mass (Da) | 15,499 | 15,566 | 16,203   | 16,129   |

* ND, not determined.

Species of the Antarctic family Artedidraconidae have only one Hb (26–29) in the adult stage. This is the first report of the complete primary structure of the single Hbs of two artedidraconid species, $A$. orianae and $P.$ scotti. A very high degree of sequence identity (96% for the $\alpha$ chains, and 90% for the $\beta$) was found between the Hbs of $A$. orianae and $P.$ scotti (Table V), higher than the identity with the major Hbs of species belonging to other Antarctic families (82–91 and 77–83%, respectively). As usual (10), the identity with minor Hbs (Hb 2 and Hb C) of Antarctic fish and with Hbs of non-Antarctic species was substantially lower (51–68%). Although cladograms (6, 30) indicate the family Bathydraconidae to be evolutionarily farther apart from Nototheniidae than Artedidraconidae, the artedidraconid sequences show lesser identity with those of two nototheniid species ($T.$ newnesi and Trematomus bernacchii) than the identity between the nototheniids and two bathydraconids (Gymnodraco acuticeps and Cygnodraco mawsoni). Sequences of Hbs of other notothe-
noids, together with evidence from phylogenetic analysis based on partial sequences of 12 S and 16 S mitochondrial ribosomal RNA (31), will hopefully contribute to understanding the evolutionary history.

Among the functionally important amino acid residues (32), in the \( \beta \) chain of both Hbs, Ser F9, Glu FG1, Gln HC1, and His HC3 are conserved, Arg H21 is conservatively replaced by Lys, whereas Asp NA2 and Lys EF6 are replaced by Gln and Met, respectively. At the \( \alpha_{1}\beta_{2} \) interface, the residues forming the flexible joint between the \( \alpha_{1} \) FG corner and the \( \beta_{2} \) C helix (Arg \( \beta_{6} \), Trp \( \beta_{3} \), Arg aFG4, Asp aG1, and Pro aG2) are conserved; among the residues forming the switch region between the \( \alpha_{1} \) C helix and the \( \beta_{2} \) FG corner, His \( \beta_{4} \) G4 and Thr \( \alpha_{6} \) C6 are conserved, whereas Thr \( \alpha_{3} \) C3 and Pro aC2 are replaced by Gln (as in all fish hemoglobins) and Ala (as in Cyprinus carpio and Catostomus clarkii), respectively.

The Hbs of \( A.\ oriana \) and \( P.\ scott \) (as well as those of other species of Artedidraconidae, such as Artedidraco shackletoni, D. longedorsalis, Pagonophryne sp. 1, sp. 2 and sp. 3)\(^2\,\,\,\,3\) are characterized by a modest Bohr effect, very weak or no Root effect, and very low cooperativity of oxygen binding. Similar results were obtained with blood, intact erythrocytes, or unstripped hemolysates. ATP slightly enhances the Bohr effect, and induces the Root effect to a limited extent. The Root effect is further induced upon addition of ATP to blood, intact erythrocytes, or unstripped hemolysates, even in specimens that had recovered from the stress of capture, suggesting that the organic phosphate is never in the erythrocytes at saturating concentrations. This similarity in oxygen-binding behavior confirms previous findings on other Antarctic fish Hbs (33–35).

The study of the regulation of the oxygen affinity by temperature in \( A.\ oriana \) Hb clearly indicates a marked temperature dependence of the Bohr effect, which is enhanced when the temperature is lowered, underlying an efficient coupling of oxygen and proton transport only at very low temperatures. In particular, very high values of oxygenation enthalpy are measured at alkaline pH values.

These observations deserve some comments, at both functional and structural level. The \( \alpha \) and \( \beta \) chains of the two Hbs show a substitution at position B10. This residue (Leu in all the

\(^2\) G. di Prisco, unpublished data.

\(^3\) M. Tamburrini and G. di Prisco, unpublished data.
The dissociation curve is hyperbolic (Fig. 9) rather than sigmoidal, and the very low levels, or lack, of subunit cooperativity. The lack of Bohr and Root effects in A. orianae, although limited, could be enough to account for P. scotti and thus for their functional dynamic heterogeneity.

Furthermore, this substitution, which occurs on the distal side of the heme pocket, might be at least partially responsible for the different kinetic behavior displayed by the two subunits, of the proximal side is similar to that of other mammalian hemoproteins, and that, probably, the lower binding rate constant observed in most other hemoproteins, including human HbA (24, 25), indeed suggests that in both fish Hbs the conformational transition upon going from the deoxy to the oxy state. This question on such a crucial point of the structure-function relationship of A. orianae Hb remains open. The oxygen-transport system of these fish has strong functional similarities with that of a Triassic reptilian relict, Sphenodon punctatus (38, 39); the exceptionally low resting meta-

The subunit heterogeneity upon oxygen dissociation in A. orianae Hb, although limited, could be enough to account for the very low levels, or lack, of subunit cooperativity. The lack of cooperativity of A. orianae Hb implies that the Hb-oxygen dissociation curve is hyperbolic (Fig. 9) rather than sigmoidal, thereby not allowing large volumes of oxygen to be bound or released in response to small changes in the blood oxygen partial pressure. The data reported in Fig. 9 are well described by the Hill plot (see inset). The Hill coefficient, at pH 8.0, is 1.01 at 10 °C and 0.81 at 2 °C, suggesting that the Hb of A. orianae, in temperature conditions close to the physiological ones, is actually characterized by negative cooperativity of ligand binding. These observations, together with the high values of oxygen enthalpy measured under alkaline conditions, raise the question whether or not this Hb undergoes the T → R conformational transition upon going from the deoxy to the oxy state. This question on such a crucial point of the structure-function relationship of A. orianae Hb remains open.

The oxygen-transport system of these fish has strong functional similarities with that of a Triassic reptilian relict, Sphenodon punctatus (38, 39); the exceptionally low resting meta-

| Species | C. carpio* | S. irideusa* | T. newnesi* | N. coriiceps | A. orianae | P. scotti | G. acuticeps | C. mawsoni | T. bernacchii | T. newnesi |
|---------|------------|--------------|-------------|--------------|------------|------------|-------------|------------|-------------|-----------|
| α Chains |            |              |             |              |            |            |             |            |             |           |
| N. coriiceps Hb 1 | 59 | 57 | 55 | 61 | 63 | 91 | 91 | 82 | 83 | 89 | 87 |
| T. newnesi Hb 1, Hb C | 58 | 62 | 52 | 63 | 66 | 86 | 85 | 92 | 90 | 97 |
| T. bernacchii Hb 1 | 64 | 62 | 57 | 65 | 70 | 87 | 88 | 91 | 91 |
| C. mawsoni Hb 1, Hb 2 | 60 | 62 | 53 | 64 | 69 | 84 | 85 | 93 |
| G. acuticeps | 58 | 62 | 53 | 65 | 67 | 82 | 82 |     |     |     |     |
| P. scotti | 66 | 60 | 60 | 65 | 68 | 96 |     |     |     |     |     |
| A. orianae | 67 | 61 | 60 | 64 | 68 |     |     |     |     |     |     |
| N. coriiceps Hb 2 | 63 | 63 | 62 | 93 |     |     |     |     |     |     |     |
| T. newnesi Hb 2 | 61 | 58 | 62 |     |     |     |     |     |     |     |     |
| Salmo irideus* Hb 1 | 66 | 60 |     |     |     |     |     |     |     |     |     |
| S. irideusa* Hb IV | 63 |     |     |     |     |     |     |     |     |     |     |

| Species | C. carpio* | S. irideusa* | T. newnesi* | N. coriiceps | A. orianae | P. scotti | G. acuticeps | C. mawsoni | T. bernacchii | T. newnesi |
|---------|------------|--------------|-------------|--------------|------------|------------|-------------|------------|-------------|-----------|
| β Chains |            |              |             |              |            |            |             |            |             |           |
| N. coriiceps Hb 1, Hb 2 | 57 | 63 | 53 | 65 | 70 | 83 | 82 | 80 | 88 | 90 | 86 |
| T. newnesi Hb 1, Hb 2 | 57 | 62 | 53 | 64 | 68 | 79 | 77 | 80 | 84 | 93 |
| T. bernacchii Hb 1 | 61 | 66 | 58 | 66 | 70 | 80 | 79 | 83 | 87 |
| C. mawsoni Hb 1 | 56 | 62 | 53 | 67 | 70 | 81 | 83 | 85 |
| G. acuticeps | 56 | 59 | 55 | 65 | 67 | 77 | 78 |     |     |     |     |
| P. scotti | 56 | 60 | 51 | 62 | 65 | 90 |     |     |     |     |     |
| A. orianae | 59 | 62 | 54 | 64 | 68 |     |     |     |     |     |     |
| T. newnesi Hb C | 57 | 62 | 57 | 89 |     |     |     |     |     |     |     |
| C. mawsoni Hb 2 | 55 | 60 | 54 |     |     |     |     |     |     |     |     |
| S. irideus* Hb I | 64 | 59 |     |     |     |     |     |     |     |     |     |
| S. irideus* Hb IV | 73 |     |     |     |     |     |     |     |     |     |     |

Non-Antarctic species.
bolic rate and low temperature of a primitive vertebrate is considered to be consistent with reduced Bohr effect, absence of cooperative oxygen binding, and high oxygen affinity (decreased by ATP with no effect on cooperativity) displayed by the hemolysate and Hbs of this organism. These remarkable characteristics are shared by the Hbs of *A. orianae* and *P. scotti*. Are these hematological features a result of life-style adaptation to extreme conditions, or did “deleterious” Hb mutations force these fish to migrate to a low-temperature habitat? This question remains open. In fact it is difficult to establish consensus on objective criteria to identify a phenotypic trait as an adaptation, which remains a “slippery concept” (40, 41). However, it is astonishing that the Hbs of Artedidraconidae (which are not primitive vertebrates) display functional properties typical of primitive organisms. Bearing in mind that Antarctic fishes have very low metabolic rates and decreased dependence on Hb-mediated oxygen transport (10, 26) and that in fact the blood of Channichthyidae, the most phyletically derived nonthetnoid family, is devoid of Hb (4), this feature may be correlated to the less critical role of Hb in sluggish Artedidraconidae. In these fishes, the Hb physiological role might merely be that of an “oxygen store” when the animal encounters anoxic conditions.

The high sequence identity of major Hbs of other notothenioids (which show cooperative interactions) with the Hbs of Artedidraconidae induces to consider the latter Hbs as “modern.” As a consequence of the above arguments, interesting questions arise on the evolution and mode of function of multisubunit molecules.

Acknowledgments—This study is in the framework of the Italian National Program for Antarctic Research. G. di Prisco and M. Tamburrini are grateful to the Alfred Wegener Institute, Bremerhaven, Germany, for the invitation to participate in the expedition Ant X/3 (March-May 1992), in the Northeastern Weddell Sea. We thank the captain and crew of the RV “Polarstern” for logistic support in field operations. We also thank Dr. L. Camardella for mass spectrometry measurements.

REFERENCES

1. Everson, I., and Ralph, R. (1968) Bull. Br. Antarct. Surv. 15, 59–62
2. Hureau, J.-C., Petit, D., Fine, J. M., and Marneux, M. (1977) in Adaptations within Antarctic Ecosystems (Llano, G. A., ed) pp. 459–477, Smithsonian Institution, Washington, D. C.
3. Wells, R. M. G., Ashby, M. D., Duncan, S. J., and Macdonald, J. A. (1980) J. Fish Biol. 17, 517–527
4. RUSD, J. T. (1954) Nature 173, 848–850
5. Gon, O., and Heemstra, P. C. (eds) (1990) Fishes of the Southern Ocean, JLB Smith Institute of Ichthyology, Grahamstown, South Africa
6. Eastman, J. T. (1983) Antarctic Fish Biology: Evolution in a Unique Environment, Academic Press, San Diego
7. Baluskin, A. V. (1992) J. Ichthyol. 30, 132–147
8. di Prisco, G., and D’Avino, R. (1989) Antartic. Sci. I, 119–124
9. di Prisco, G., D’Avino, R., Camardella, L., Caruso, C., Romano, R., and Rutigliano, B. (1990) Polar Biol. 10, 269–274
10. di Prisco, G., D’Avino, R., Caruso, C., Tamburrini, M., Camardella, L., Rutigliano, R., Carratore, V., and Romano, M. (1991) in Biology of Antarctic Fish (di Prisco, G., Maresca, B., and Tota, B., eds) pp. 263–281, Springer-Verlag, Berlin
11. di Prisco, R., Caruso, C., Tamburrini, M., Romano, R., Rutigliano, R., Polverino de Laureto, P., Camardella, L., Carratore, V., and di Prisco, G. (1994) J. Biol. Chem. 269, 9675–9681
12. Andriashev, A. P. (1965) Monogr. Biol. 25, 491–550
13. D’Avino, R., and di Prisco, G. (1988) Comp. Biochem. Physiol. B Comp. Biochem. 90, 579–584
14. Laemml, U. K. (1970) Nature 227, 680–685
15. D’Avino, R., and di Prisco, G. (1989) Eur. J. Biochem. 179, 699–705
16. D’Avino, R., Caruso, C., Romano, M., Camardella, L., Rutigliano, R., and di Prisco, G. (1989) Eur. J. Biochem. 179, 707–713
17. Tamburrini, M., Branjacce, A., Ippoliti, R., and di Prisco, G. (1992) Arch. Biochem. Biophys. 292, 295–302
18. Brauer, A., Oman, C. L., and Margolies, M. N. (1984) Anal. Biochem. 137, 134–142
19. Giardina, B., and Amiconi, G. (1981) Methods Enzymol. 76, 417–427
20. Root, R. W. (1951) Biol. Bull. (Woods Hole) 61, 427–456
21. Brittain, T. (1987) Comp. Biochem. Physiol. B Comp. Biochem. 86, 473–481
22. Coletta, M., Ascenzi, P., Smulewich, G., Mantine, A. R., Del Gaudio, R., Piscopo, M., and Geraci, G. (1992) FEBS Lett. 296, 184–186
23. Fago, A., Romano, M., Tamburrini, M., Coletta, M., D’Avino, R., and di Prisco, G. (1993) Eur. J. Biochem. 218, 829–833
24. Coletta, M., Ascenzi, P., and Brunori, M. (1988) J. Biol. Chem. 263, 18286–18289
25. Coletta, M., Ascenzi, P., Traylor, T. G., and Brunori, M. (1985) J. Biol. Chem. 260, 4151–4155
26. di Prisco, G., Caruso, C., Tamburrini, M., Romano, M., Rutigliano, R., and di Prisco, G. (1993) Fishes of the Southern Ocean, Cambridge University Press, Cambridge, pp. 517–517
27. di Prisco, G., and Giardina, B. (1996) Soc. Exp. Biol. Semin. Ser. 59, 23–51
28. di Prisco, G. (1997) in Proceedings of the SCAR 6th Biological Symposium, Venice (Antarctic Communities: Structure and Survival) (Battaglia, B., Valencia, J., and Walton, D. W., eds) pp. 251–260, Cambridge University Press, Cambridge
29. di Prisco, G., Tamburrini, M., and D’Avino, G. (1997) Soc. Exp. Biol. Semin. Ser. 66, 143–165
30. Iwami, T. (1985) Mem. Nat. Inst. Polar Res. Ser. E Biol Med. Sci. 36, 1–69
31. Bargelloni, L., Ritchie, P. A., Patarnello, T., Battaglia, B., Lambert, D. M., and Meyer, A. (1994) Mol. Biol. Evol. 11, 854–863
32. Perutz, M. F., and Brunori, M. (1982) Nature 299, 421–426
33. di Prisco, G. (1988) Comp. Biochem. Physiol. B Comp. Biochem. 90, 631–637
34. di Prisco, G., Giardina, B., D’Avino, R., Condo, S. G., Belgelli, A., and Brunori, M. (1988) Comp. Biochem. Physiol. B Comp. Biochem. 95, 585–591
35. di Prisco, R., and di Prisco, G. (1987) Eur. J. Biochem. 179, 699–705
36. Kloek, A. P., Yang, J., Mathews, F. S., Frieden, C., and Goldberg, D. E. (1994) J. Biol. Chem. 269, 2377–2379
37. Yang, J., Kloek, A. P., Goldberg, D. E., and Mathews, F. S. (1995) Comp. Biochem. Physiol. B Comp. Biochem. 109, 473–481
38. Wells, R. M. G., Tetens, V., and Brittain, T. (1983) Nature 306, 500–502
39. Tetens, V., Brittain, T., Christie, D. L., Robb, J., and Wells, R. M. G. (1984) Comp. Biochem. Physiol. B Comp. Biochem. 79, 119–123
40. Reeve, H. K., and Sherman, P. W. (1993) Quart. Rev. Biol. 68, 1–32
41. Garland, T., Jr., and Carter, P. A. (1994) Annu. Rev. Physiol. 56, 579–621
The Hemoglobins of the Antarctic Fishes Artedidraco oriana and Pogonophryne scotti: AMINO ACID SEQUENCE, LACK OF COOPERATIVITY, AND LIGAND BINDING PROPERTIES

Maurizio Tamburrini, Mario Romano, Vito Carratore, Andreas Kunzmann, Massimo Coletta and Guido di Prisco

J. Biol. Chem. 1998, 273:32452-32459.
doi: 10.1074/jbc.273.49.32452

Access the most updated version of this article at http://www.jbc.org/content/273/49/32452

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 5 of which can be accessed free at http://www.jbc.org/content/273/49/32452.full.html#ref-list-1