The Primary Structure of Minke-Whale(Balenoptera acutorosterata-Cetacea) Hemoglobin

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Summary: The complete primary structure of Minke-Whale hemoglobin has been determined. Separation of the chains was carried out by ion-exchange chromatography on CM-Cellulose in 8M urea, the sequence was analysed in liquid phase sequenator. Alignment was done in homology with Dolphin (Tursiops truncatus) hemoglobin sequence. There are 14 and 12 amino acid exchanges in α- and β-chain respectively in comparison with Dolphin. Besides, the α and β-chains showed a single peak in chromatography. There are many ambiguities in the sequence (2 in α- and 4 in β-chains, all neutral exchanges) showing the polymorphic nature of globin α- and β-genes.

Introduction

Hemoglobin, the respiratory blood pigment found in vertebrates and to a certain extent in invertebrates, is the best known protein. The vast interest in its study stems from the realization of the fact that it exhibits relationship within and among the different group of organisms which in turn shows not only the phylogenetic aspect of their origin but also the course of their adaption.

Among vertebrates, mammals have been the subject of special interest. Whales, the marine group of vertebrates, were classified for the first time by E.J.Ray in 1693 as "Mammals". Thus, the order "Cetacea" comprises of these marine mammals. We have attempted to study the hemoglobin of Whale belonging to the family "Balenoptera" and in this communication the amino acid sequence of α- and β-chains from Balenoptera acutorosterata is presented.

Experimental

Materials and Methods:

Blood was obtained from a male with a body length of 6.9 m (gift of Prof. G.Matsuda, Nagasaki). Erythrocytes were separated by centrifugation and washed thrice with physiological saline. Cells were lysed with bidistilled water, globin was prepared from the whole hemolysate according to the method of

Abbreviations: Hb = Hemoglobin: Reagent I = 4-(Isothiocyanato)benzenesulphonic acid. Sodium salt; Reagent IV = 7-(Isothiocyanato)naphthalene-1,3,5-trisulphonic acid, trisodium salt; Quadrol = N,N,N',N'-tetrakis-(2-hydroxypropyl)ethylenediamine.

Rossi-Fanelli and Antonini [1]. Globin chains were separated by ion-exchange chromatography on CM-Cellulose [2] (Whatman CM-52) column (15 x 2.6 cm) in 8M urea, containing 0.2% Mercaptoethanol and 0.025M sodium acetate at pH 5.6, with a sodium chloride gradient of 0 - 0.1M or 8M urea, containing 0.2% mercaptoethanol and phosphate gradient of 0.001M (equilibrating buffer) to 0.005M at a constant pH of 6.8.

Electrophoresis

Whole hemolysate was analysed by polyacrylamide disc electrophoresis [3] using 10% gels and Tris-glycine buffer, pH 8.3, Globin chains from CM-Cellulose chromatography were analysed by polyacrylamide gel electrophoresis in presence of 8M urea and Triton X-100 according to Alter et al.[4].

Enzymic and chemical cleavage

Peptides were obtained by digestion of globin chains with 5% trypsin (TPCK-treated). Overlapping peptides were obtained by limited tryptic digestion, performed on globin chains after blocking ε-lysyl groups with citraconic anhydride [5]. C-terminal peptides were obtained by cleavage at Asp-Pro [6] bond with 70% formic acid in presence of 6M guanidine hydrochloride.

Separation of peptides

Prefractionation of peptides was carried out by gel filtration. Peptides from tryptic digestion were fractionated on Sephadex G-25 (fine) using 0.1N acetic acid whereas those from limited tryptic digestion were fractionated on Sephadex G-50 (fine). Hydrolytic peptides were separated on Sephadex G-50 (fine) using 8M urea in 10% formic acid. Rechromatography was then carried out on HPLC employing RP-2 column with 50 mM ammonium acetate [7] and acetonitrile gradient of 0 - 60%. In some cases purification was carried out on Vydac RP18 using 0.1% TFA and acetonitrile gradient of 0 - 65%.

Amino acid analysis

Samples were hydrolysed in constant boiling HCl (5.7N) at 110°C for 20 hours. Analysis was performed on a Biotronic autoanalyser. Cysteine and methionine were determined after oxidation with performic acid. Tryptophan was estimated after hydrolysis in presence of 6% thioglycollic acid.

Sequence determination

Sequence studies were carried out by automatic Edman degradation using Beckman sequencers. Intact globin chain or peptides were sequenced using either Quadrol [8] (0.25 or 1.0M) or Propyne program [9]. Lysine peptides were modified with reagent I [10] or IV [11] prior to sequencing. PTH derivatives of amino acids were identified by thin layer chromatography [9] on Silica gel plates (E.Merck precoated) or on HPLC [12].

Results and Discussion

Disc electrophoresis of the hemolysate showed the presence of a single component. Chromatography on CM-Cellulose correspondingly yielded only two peaks. However, polyacrylamide gel electrophoresis in presence of 8M urea and Triton X-100 revealed the presence of two equally intense bands identified as α chains. Heterogenity was similarly observed for the peak identified as β-chains. Tryptic digestion from this preparation also confirmed the existence of two different α-chains differing at position α 21 by Gly/Ser and at position α96 by Ala/Val. Positions 72, 87, 123 and 128 were found to be heterogeneous for β-chains with β 72 Ser/Ala, β 87 Thr/Ala, β 123 Thr/Leu and β 128 Ala/Ser. The ratio

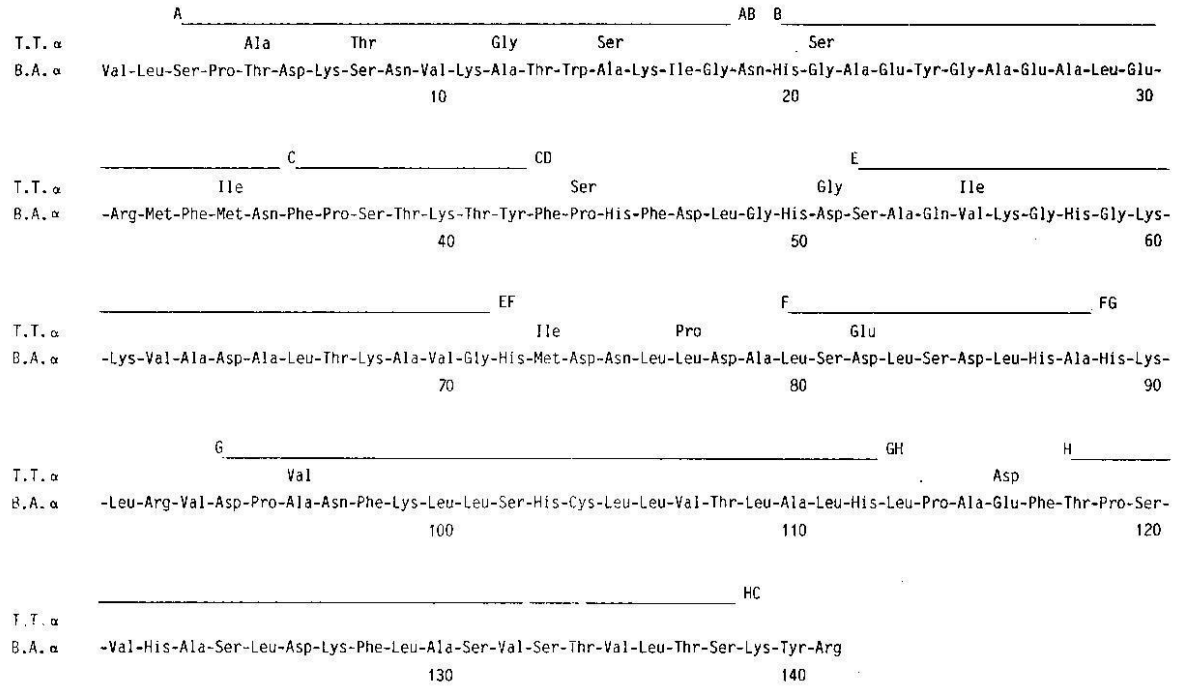

Fig.1: The amino-acid sequence of α-chain from Minke-Whale hemoglobin (Balenoptera acutorosterata) in homologous alignment with Dolphin hemoglobin (Tursiops truncatus) α-chain.

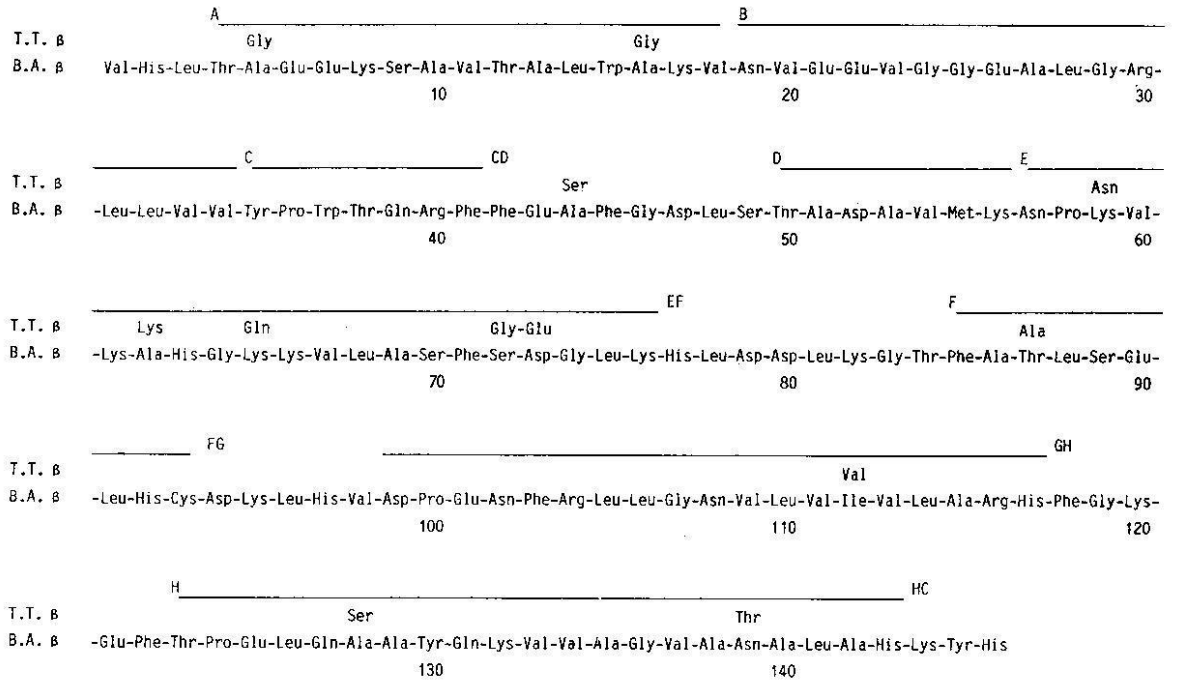

Fig.2: The amino-acid sequence of β-chain from Minke-Whale hemoglobin (Balenoptera acutorosterata) in homologous alignment with Dolphin hemolgobin (Turiops truncatus) β-chain.

of PTH amino acids was found to be 1:1 .

The complete sequence of α- and βchain is presented in Fig.1 and 2. A comparison of the sequence with that of Dolphin (T.truncatus, Cetacea) shows 14 substitutions in the α- and 12 substitutions in the β-chains accounting for 14 base pair exchanges in the case of α-chains and 13 base pair exchanges in the case of β-chain.

The structural and functional importance of these exchanges with respect to respiration physiology will be discussed elsewhere.

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