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Complete Amino Acid Sequence of the Myoglobin from the Dall Porpoise (*Phocoenoides dalli dalli*) and Reinvestigation of the Primary Structure of the Myoglobin from Common Porpoise (*Phocoena phocoena*)†

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ABSTRACT: The complete amino acid sequence of the principal component myoglobin of the Dall porpoise, *Phocoenoides dalli dalli*, was determined by specific cleavage of the protein to obtain large peptides which are readily degraded by the automatic sequencer. The apomyoglobin was selectively cleaved at the two methionine residues with cyanogen bromide and the acetimidated apomyoglobin was cleaved at the three arginine residues by trypsin. Over 80% of the primary structure of the protein was obtained by subjecting four of these peptides and the apomyoglobin to automatic Edman degradation. The remainder of the primary structure was determined by sequence analysis of tryptic peptides isolated from the central cyanogen bromide fragment after modification of the single arginine residue with 1,2-cyclohexanedione as well as by staphylococcal protease digestion of the same unmodified cyanogen bromide fragment. This myoglobin differs from that of sperm whale, *Physeter catodon*, at 15 positions and from the myoglobins of various members of the closely related family *Delphinidae* (dolphins) at no more than six positions. Also reported in this paper is evidence that the amino acid sequence of the myoglobin of the common porpoise, *Phocoena phocoena*, is identical with that of the Dall porpoise instead of having two residue substitutions as previously determined.

This paper reports the use of the automatic Edman degradation procedure in determining the complete amino acid sequence of the myoglobin from the Dall porpoise, *Phocoenoides dalli dalli*. The peptide fragmentation and the analytical procedures necessary for this determination have been established in several other cetacean myoglobin sequence papers (Dwulet et al., 1975, 1977; Bogardt et al., 1976; Jones et al., 1976, 1978; Lehman et al., 1977; Wang et al., 1977; DiMarchi et al., 1978). The sequence reported here differs in only two residue positions from that reported for the common porpoise, *Phocoena phocoena* (Bradshaw & Gurd, 1969). A direct comparison by identical methods of the properties of samples of these proteins available to us has failed to show any difference between the myoglobins of these two species and raises the question of a possible error in the previous determination of the common porpoise sequence.

Experimental Section

Materials. All materials were as described in Dwulet et al. (1977) except that the myoglobin preparation involved purification on CM-50 Sephadex in phosphate buffer, ionic strength 0.1 at pH 6.8 instead of pH 6.4.

Methods. All chemical modifications, enzymatic and chemical cleavages, and peptide fractionation procedures were as described previously (Dwulet et al., 1975; Bogardt et al., 1976; Jones et al., 1978).

Results

Amino Acid Composition. The amino acid composition of the principal component of Dall porpoise myoglobin was obtained from 24, 48, and 72 h hydrolysates of the apomyoglobin. The results are summarized in Table I.

Peptide Separation. All generated peptides were purified as described in the preceding papers in this series. The fractionation profiles and peptide compositions are presented in the supplementary material (see paragraph concerning supplementary material at the end of this paper).

Sequence Investigations. Only the sequence data necessary to establish the entire primary structure are reported.

Sequencer Results. The complete primary structure of Dall

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                    5                   10
 1  Gly Leu Ser Glu Gly Glu Trp Gln Leu Val Leu Asn Val Trp Gly
16  Lys Val Glu Ala Asp Leu Ala Gly His Gly Gln Asp Val Leu Ile
31  Arg Leu Phe Lys Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys
46  Phe Lys His Leu Lys Thr Glu Ala Glu Met Lys Ala Ser Glu Asp
61  Leu Lys Lys His Gly Asn Thr Val Leu Thr Ala Leu Gly Gly Ile
76  Leu Lys Lys Lys Gly His His Asp Ala Glu Leu Lys Pro Leu Ala
91  Gln Ser His Ala Thr Lys His Lys Ile Pro Ile Lys Tyr Leu Glu
106 Phe Ile Ser Glu Ala Ile Ile His Val Leu His Ser Arg His Pro
121 Ala Glu Phe Gly Ala Asp Ala Gln Gly Ala Met Asn Lys Ala Leu
136 Glu Leu Phe Arg Lys Asp Ile Ala Thr Lys Tyr Lys Glu Leu Gly
151 Phe His Gly
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FIGURE 1: The complete amino acid sequence of Dall porpoise myoglobin.

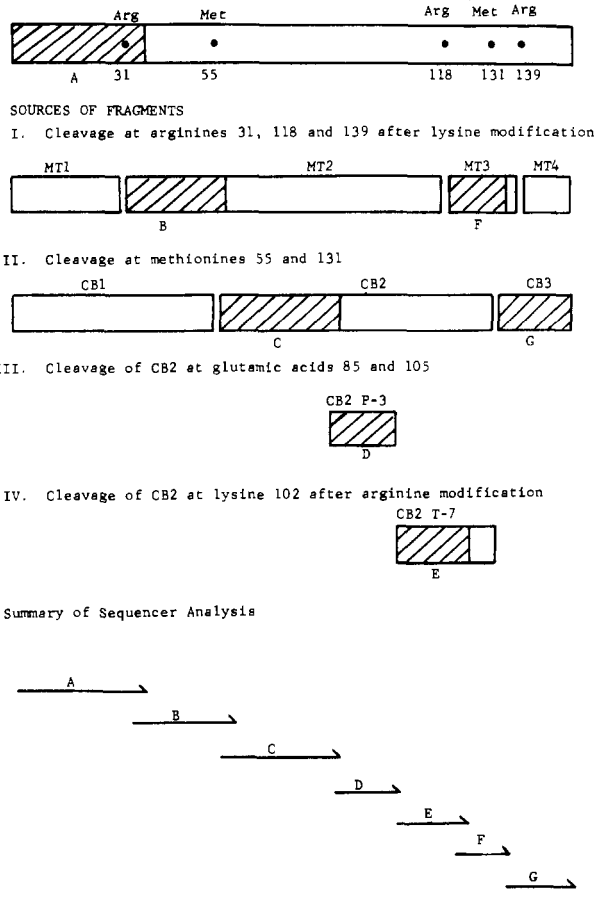

FIGURE 2: Summary of the peptides prepared from Dall porpoise myoblobin for sequencer analysis. The top bar represents the whole protein and the residues important for its fragmentation. A hatched section in each bar indicates the segment of sequence determined by sequence analysis on that fragment: (A) 1-35; (B) 32-59; (C) 56-89; (D) 86-105; (E) 103-122; (F) 119-135; (G) 132-153.

porpoise myoglobin is shown in Figure 1. The sequence strategy used is outlined diagrammatically in Figure 2.

Discussion

Comparison with Sequence of Common Porpoise Myoglobin. The reported sequence for common porpoise myoglobin

TABLE I: Amino Acid Composition of *Phocoenoides dalli dalli* Myoglobin.

amino acid	from acid hydrolysates[a]	from the sequence
Asp	10.4	10
Thr	6.2	6
Ser	4.8	5
Glu	17.8	18
Pro	4.1	4
Gly	14.3	14
Ala	15.3	15
Val	6.4	6
Met	1.8	2
Ile	7.2	8
Leu	18.8	19
Tyr	1.6	2
Phe	6.5	7
Lys	20.2	20
His	11.9	12
Arg	2.6	3
Trp[b]	2.2	2

[a] Acid hydrolyses were performed on apomyoglobin for 24, 48, and 72 h at 110 °C with 5.7 N HCl and the values were averaged. The amino acid residues were calculated on the basis of 153 amino acids in the protein. The value for serine was obtained by extrapolation to zero time. The value for isoleucine was the maximum value (72 h). [b] Tryptophan was determined by the method of Liu & Chang (1971).

(Bradshaw & Gurd, 1969) differs from that of Dall porpoise myoglobin at two residue positions, 83 and 85. Common porpoise myoglobin was found to contain a glutamic acid residue at position 83 and an asparagine residue at position 85, in contrast to Dall porpoise myoglobin (Figure 1) which has an aspartic acid residue and a glutamic acid residue, respectively, at these two positions. Because of the amide at position 85 the common porpoise myoglobin should have a more positive net charge than Dall porpoise myoglobin and, therefore, should have a higher isoionic (p*I*) value. However, the p*I* of Dall porpoise myoglobin was found to be 8.28, which is indistinguishable from the value of 8.26 (Hartzell et al., 1968) obtained with the protein from the same source as that used for the earlier sequence work (Bradshaw & Gurd, 1969).

In an attempt to resolve this discrepancy the sequence of common porpoise myoglobin was reinvestigated. The apomyoglobin from freshly prepared common porpoise myoglobin (principal component) was cleaved with cyanogen bromide and the various peptide fragments were isolated (see supplementary material). The cyanogen bromide middle fragment (CB2: 56-131) was then subjected to automated Edman degradation through position 90. All positions including the aspartic acid at position 83 and the glutamic acid at position 85 were identical with those of Dall porpoise myoglobin. Furthermore, the freshly isolated common porpoise myoglobin had the same electrophoretic properties as Dall porpoise myoglobin on cellulose-acetate electrophoresis at pH 9.2, and its p*I* value was also identical at 8.28, which is again not significantly different from the earlier reported value of 8.26. A further check proved possible by direct analysis of the chymotryptic pool XI-XII-3 (peptide C-13) that had been used in the previous study (Bradshaw & Gurd, 1969). This was one of two pools employed to define the sequence of residues 77 through 89. The overall composition was found to conform to the earlier analysis. The peptide was subjected to automated Edman degradation according to the peptide protocol (see Methods). The presence of the dicarboxymethylated histidine residues at positions 81 and 82 (Bradshaw & Gurd, 1969) led

Residue Number	1	4	12	13	15	21	28	35	45	54
Dall Porpoise (Phocoenidae)	Gly	Glu	Asn	Val	Gly	Leu	Val	Gly	Lys	Glu
Common Porpoise (Phocoenidae)	Gly	Glu	Asn	Val	Gly	Leu	Val	Gly	Lys	Glu
Bottlenosed Dolphin (Delphinidae)	Gly	Asp	Asn	Val	Gly	Leu	Val	Gly	Lys	Asp
Common Dolphin (Delphinidae)	Gly	Asp	Asn	Val	Gly	Leu	Val	Gly	Lys	Asp
Pilot Whale (Delphinidae)	Gly	Asp	Asn	Val	Gly	Leu	Ile	Gly	Lys	Asp
Killer Whale (Delphinidae)	Gly	Asp	Asn	Val	Gly	Leu	Ile	Gly	Lys	Asp
Amazon River Dolphin (Platanistidae)	Gly	Asp	Asn	Ile	Gly	Leu	Val	Gly	Lys	Glu
Sperm Whale (Physeteridae)	Val	Glu	His	Val	Ala	Val	Ile	Ser	Arg	Glu

Residue Number	66	74	83	85	121	122	129	144	151	152
Dall Porpoise (Phocoenidae)	Asn	Gly	Asp	Glu	Ala	Glu	Gly	Thr	Phe	His
Common Porpoise (Phocoenidae)	Asn	Gly	Asp	Glu	Ala	Glu	Gly	Thr	Phe	His
Bottlenosed Dolphin (Delphinidae)	Asn	Ala	Asp	Glu	Ala	Glu	Gly	Ala	Phe	His
Common Dolphin (Delphinidae)	Asn	Ala	Asp	Glu	Ala	Glu	Gly	Ala	Phe	His
Pilot Whale (Delphinidae)	Asn	Ala	Glu	Glu	Ala	Glu	Gly	Ala	Phe	His
Killer Whale (Delphinidae)	Asn	Ala	Asp	Glu	Ala	Gln	Gly	Ala	Phe	His
Amazon River Dolphin (Platanistidae)	Asn	Gly	Glu	Glu	Gly	Asp	Ala	Ala	Phe	His
Sperm Whale (Physeteridae)	Val	Ala	Glu	Glu	Gly	Asp	Gly	Ala	Tyr	Gln

FIGURE 3: Comparison of the amino acid sequences of various cetacean myoglobins. Family names are shown in parentheses. Only those positions in which differences occur, apart from position 85, are reported. All other positions are the same as in the Dall porpoise myoglobin sequence. Assignments at positions 83 and 85 of the common porpoise myoglobin sequence are as corrected in this paper (see text). The reassignment of position 122 of the sperm whale myoglobin sequence is due to Romero-Herrera & Lehmann (1974).

to a very low yield in subsequent rounds. The gas–liquid chromatography identifications were indicative rather than conclusive, but it was possible by thin-layer chromatography to obtain clear evidence for aspartic acid at position 83 and some indication of glutamic acid at position 85. It would appear, therefore, that the myoglobins of the common porpoise and of the Dall porpoise have identical sequences rather than the reported two differences.

Comparison with Sequence of Pilot Whale Myoglobin. As seen in Figure 3 Dall porpoise myoglobin has close similarities in sequence with various members of the family *Delphinidae* (dolphins). Dall porpoise myoglobin differs in sequence by only four residues from the identical sequences of the Atlantic bottlenosed dolphin (Jones et al., 1976) and Pacific common dolphin (Wang et al., 1977) myoglobins and by six residues from the sequences of pilot whale myoglobin (Jones et al., 1978) and killer whale myoglobin (Castillo et al., 1977). The sequence of Dall porpoise myoglobin will be compared here with that of pilot whale myoglobin. The differences between the two sequences will be referred to by first giving the position number, then the residue found in Dall porpoise myoglobin, followed by the homologous pilot whale residue in parentheses.

Position 4 Glutamic Acid (Aspartic Acid). For the majority of known myoglobins aspartic acid is the common residue here. Among Cetacea only the myoglobins of Dall porpoise, common porpoise, sperm whale (Edmundson, 1965), and dwarf sperm whale (Dwulet et al., 1977) contain a glutamic acid at this position.

Position 28 Valine (Isoleucine). Valine is the common amino acid found at this position for most mammalian myoglobins but isoleucine is found in the majority of Cetacea.

Position 54 Glutamic Acid (Aspartic Acid). The common residue at position 54 is glutamic acid. The myoglobins of Black Sea dolphin (Kluh & Bakardjieva, 1971), killer whale, bottlenosed dolphin, and common dolphin are the only other Cetacea to have aspartic acid at this position.

Position 74 Glycine (Alanine). The majority of myoglobins have glycine at this position, but about half of the Cetacea have alanine.

Position 83 Aspartic Acid (Glutamic Acid). This position in myoglobin is usually occupied by glutamic acid, but most dolphins and porpoises have aspartic acid.

Position 144 Threonine (Alanine). Alanine is normally the residue at this position in myoglobin. The threonine substitution is unique to Dall porpoise and common porpoise myoglobins.

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Supplementary Material Available

Experimental results including elution profiles, peptide compositions, and repetitive yield plots are provided (22 pages). Ordering information is given on any current masthead page.

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