does not combine with phalloidin and consequently is not protected by phallotoxins from proteolytic degradation.

Phalloidin exerts its protective activity of F-actin against subtilisin at least for 24 h (not measured after longer incubation times). This confirms also former observations in our laboratory that the bicyclic peptide itself is not hydrolyzed by any of numerous protein or peptide cleaving enzymes investigated. The specific protection of F-actin from proteolytic degradation enables an easy removal of non-actin proteins by treatment with proteolytic enzymes and so recovering resistant proteins from various protein mixtures.

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Complete Amino Acid Sequence of the Major Component Myoglobin of Finback Whale (*Balaenoptera physalus*)†

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ABSTRACT: The complete amino acid sequence of the major component myoglobin from finback whale, *Balaenoptera physalus,* was determined by the automated Edman degradation of several large peptides obtained by specific cleavages of the protein. Three easily separable peptides were obtained by cleaving with cyanogen bromide at the two methionine residues and one large peptide was isolated after cleavage with (2-*p*-nitrophenylsulfenyl)-3-methyl-3′-bromoindolenine. More than 60% of the covalent structure was established by the sequential degradation of three of these peptides and the apomyoglobin. An additional 30% of the primary sequence was established with peptides obtained from tryptic digestion of both the apomyoglobin and the acetimidoapomyoglobin, and the final 10% of the sequence was completed after digestion of the two larger cyanogen bromide peptides with *S. aureus* strain V8 protease. This myoglobin differs from that of the sperm whale, *Physeter catodon,* at 15 positions, from that of the arctic minke whale, *Balaenoptera acutorostrata,* at 3 positions, and from that of the California gray whale, *Eschrichtius gibbosus,* at 4 positions. All of the substitutions observed in this sequence fit easily into the three-dimensional structure of the sperm whale myoglobin.

This report presents the determination of the primary structure of the myoglobin from the finback whale, *Balaenoptera physalus.* In preceding papers, the complete amino acid sequences of several cetacean myoglobins were reported (Dwulet et al., 1975, 1977; Bogardt et al., 1976; Jones et al., 1976; Lehman et al., 1977; Wang et al., 1977). The analytical procedures developed in these papers were utilized in this sequence determination. The finback whale myoglobin was found to differ from that of the arctic minke whale, *Balaenoptera acutorostrata,* at 3 out of the 153 sequence positions.

Experimental Section

Materials

The major component of finback whale myoglobin was

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TABLE I: Amino Acid Composition of Finback Whale Myoglobin.

Amino acid	No. of residues from acid hydrolysis[a]	No. of residues from the sequence
Asp	12.3	12
Thr	5.9	6
Ser	5.1	5
Glu	16.2	16
Pro	3.9	4
Gly	10.1	10
Ala	19.3	19
Val	6.1	6
Met	2.2	2
Ile	9.6	10
Leu	17.7	18
Tyr	1.9	2
Phe	6.7	7
Lys	20.0	20
His	12.1	12
Arg	2.1	2
Trp[b]	1.9	2

[a] Samples were hydrolyzed with 5.7 N HCl in the usual manner for 24, 48, and 72 h. The values for serine and threonine are obtained by extrapolating to zero time. Isoleucine value was obtained by extrapolating to 96 h. The results obtained for all other residues were averaged. [b] Tryptophan was determined by the method of Liu & Chang (1971).

isolated from frozen muscle tissue by the procedure of Hapner et al. (1968). Phosphate buffer, pH 6.1, ionic strength 0.1, was used to effect purification of the crude homogenate on Sephadex CM-C50. The homogeneity of the purified myoglobin was affirmed by polyacrylamide gel electrophoresis at pH 9.2 and 5.2. Apomyoglobin was prepared in the presence of 0.1 M sodium fluoride as described by Yonetani (1967).

BNPS-skatole[1] was prepared by the procedures of Fontana et al. (1966) and Omenn et al. (1970). Methyl acetimidate hydrochloride was prepared according to the method of Hunter & Ludwig (1962). Preparation of 3-SPITC was by the procedure of Dwulet & Gurd (1976). Tos-PheCH_2Cl-treated trypsin was purchased from Worthington. Staphylococcal protease was obtained from Miles Laboratories Ltd. Sequencer reagents of "sequencer" grade were obtained from Beckman Instruments. All other chemicals were of the highest grade available.

Methods

Specific Enzymatic and Chemical Cleavage. Cleavage of the apomyoglobin with cyanogen bromide and the acetimidoapomyoglobin with trypsin was as described by Dwulet et al. (1975).[2] Digestion of the cyanogen bromide peptides with *S. aureus* strain V8 protease was as reported by Bogardt et al. (1976). Tryptic digestion of the apomyoglobin was as described by Dwulet et al. (1977). BNPS-skatole cleavage of the apoacetimidomyoglobin was as reported by Wang et al. (1977).

Results

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

Peptide Purification. The peptides were generated and purified as described in the preceding papers of this series. The separation 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 data necessary to establish the complete primary structure are reported.

Sequenator Results. The complete sequence of the finback whale myoglobin is presented in Figure 1. The strategy used is shown in Figure 2. The bar on the top represents the whole

```
                5                  10                  15
1   Val Leu Thr Asp Ala Glu Trp His Leu Val Leu Asn Ile Trp Ala
16  Lys Val Glu Ala Asp Val Ala Gly His Gly Gln Asp Ile Leu Ile
31  Ser 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 Glu 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 Asp Ala Ile Ile His Val Leu His Ser Arg His Pro
121 Ala Asp Phe Gly Ala Asp Ala Gln Ala Ala Met Asn Lys Ala Leu
136 Glu Leu Phe Arg Lys Asp Ile Ala Ala Lys Tyr Lys Glu Leu Gly
151 Phe Gln Gly
```

FIGURE 1: The complete amino acid sequence of finback whale myoglobin.

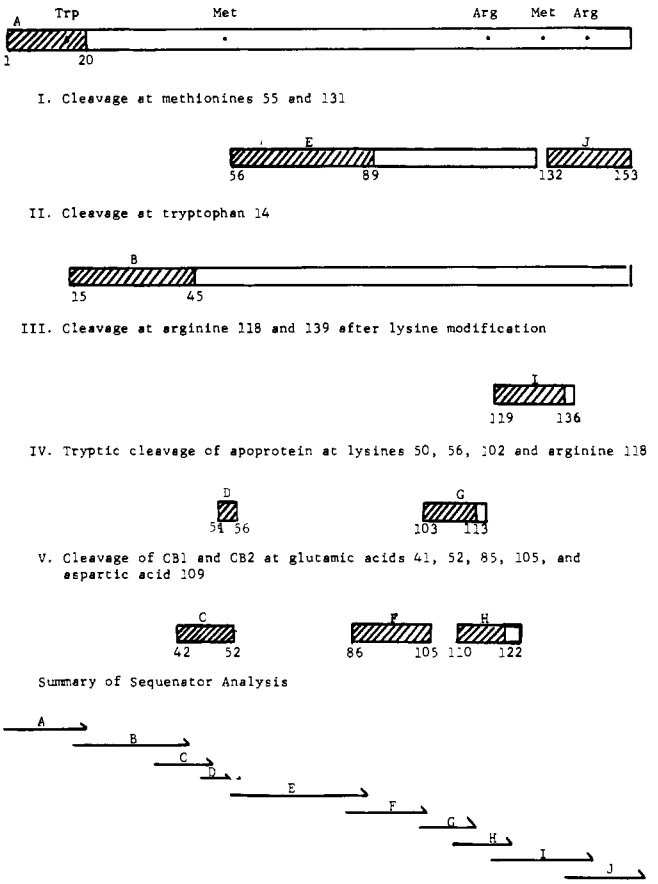

FIGURE 2: Summary of the peptides prepared from finback whale myoglobin for sequenator analysis. The number of residues analyzed in each of the hatched sections is indicated below the peptide.

[1] Abbreviations used: BNPS-skatole, (2-*p*-nitrophenylsulfenyl)-3-methyl-3′-bromoindolenine; Tos-PheCH_2Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; 3-SPITC, 3-sulfophenyl isothiocyanate.

[2] The peptide isolation profiles, peptide compositions, and repetitive yield plots of each sequenator analysis are represented in the supplementary material; see paragraph concerning supplementary material at the end of this paper.

Species	Residue Number 3	4	5	8	12	13	31	35
Sperm Whale	Ser	Glu	Gly	Gln	His	Val	Arg	Ser
Gray Whale	Ser	Asp	Ala	Gln	Asn	Ile	Arg	Gly
Minke Whale	Ser	Asp	Ala	His	Asn	Ile	Arg	Gly
Finback Whale	Thr	Asp	Ala	His	Asn	Ile	Ser	Gly

Species	Residue Number 45	66	74	109	121	122	129	151
Sperm Whale	Arg	Val	Ala	Glu	Gly	Asp	Gly	Tyr
Gray Whale	Lys	Asn	Gly	Asp	Gly	Asp	Ala	Phe
Minke Whale	Lys	Asn	Gly	Asp	Ala	Glu	Ala	Phe
Finback Whale	Lys	Asn	Gly	Asp	Ala	Asp	Ala	Phe

FIGURE 3: Comparison of the amino acid sequences of four whale myoglobins whose sequences have been completed to date. Only those positions in which differences occur are reported.

myoglobin sequence and the residues subject to the cleavage reactions are marked. The hatched sections in each bar represent the numbers of residues analyzed by the sequenator in each peptide. This strategy deviated somewhat from that described in the determination of the other cetacean myoglobin sequences. The substitution of a serine for arginine at position 31 eliminated the possibility of isolating the tryptic peptide 32–118. To compensate, the residues 32 through 55 were positioned by analysis of three other peptides (B, C, D).

Discussion

The finback whale myoglobin sequence represents the third baleen whale sequence to be completed (Bogardt et al., 1976; Lehman et al., 1977). The information derived from these sequences has been included with other known cetacean myoglobin sequences to develop a computer model of cetacean phylogenetics (Bogardt, 1978). The order Cetacea consists of two suborders commonly known as the baleen whales (suborder Mysticeti) and the toothed whales (suborder Odontoceti). The sequence of the finback whale myoglobin is compared in Figure 3 with the two other known baleen whale myoglobin sequences and with that of sperm whale myoglobin, a representative of the suborder Odontoceti. Figure 3 shows that the sequence of finback whale myoglobin is quite similar to that of the California gray and arctic minke whales while differing in almost 10% of the 153 sequence positions from that of sperm whale.

The sequence of finback whale myoglobin will be examined here in comparison to the differences in sequence from the California gray whale. These will be referred first to the residue found in finback whale myoglobin, followed by the California gray whale residue in parentheses.

Position 3 Threonine (Serine). Serine is the common residue for this position. Threonine at this position has not previously been found in any other myoglobin. The conservation of the side chain hydroxyl group suggests the involvement of this residue in a hydrogen bond formation. This conforms with the x-ray data which recognize the beginning of the α helix at residue 3 (Takano, 1977) rather than residue 4 as predicted by the general correlations of Chou & Fasman (1974).

Position 8 Histidine (Glutamine). Glutamine is the common residue for this position. The arctic minke whale is the only other cetacean myoglobin possessing a histidine at position 8.

Position 31 Serine (Arginine). Arginine is the common residue for this position. Serine at this position has not been found in any other cetacean myoglobin. The loss of a positive charge at this site should not change the tertiary structure of the molecule since the arginine is believed to lie on the surface of the molecule (Takano, 1977). This substitution is the only charge change between the arctic minke whale and finback whale myoglobins.

Position 121 Alanine (Glycine). Both alanine and glycine are common in other cetacean myoglobins at this position.

All of the above changes are compatible with the sperm whale myoglobin three-dimensional structure. No significant change in the backbone conformation of the finback whale myoglobin is expected to occur as a result of these changes.

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Supplementary Material Available

Experimental results indicating elution profiles of peptides, amino acid compositions, and sequenator repetitive yield plots are provided (27 pages). Ordering information is given on any current masthead page.

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