The Characterization of Adult Human Myoglobin

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It has been reported that crystalline human myoglobin can be resolved by electrophoresis on paper into three components (1, 2). Two of these possess nearly identical absorption spectra between 500 and 600 m\(\mu\), and give the same reactions with oxygen. Although it was suggested that these components differ structurally (2), no evidence has been presented to support this view.

In the present study the heterogeneity of human metmyoglobin has been examined with the aid of ion exchange chromatography. Five chromatographically distinct fractions, two of which represent approximately 75% of the myoglobin in skeletal muscle, have been found. Chemical and physical analyses of these fractions indicate that the globin is identical in the two myoglobins that are present in major amount. The differences in chromatographic and electrophoretic behavior of these fractions result from differences in the state of the iron in the heme prosthetic group. At least one of the other myoglobin components, which represents only a minor amount of the myoglobin of normal muscle, appears to differ in the primary structure of the globin moiety. The availability of large amounts of chromatographically pure myoglobin has prompted further characterization of this protein.

EXPERIMENTAL PROCEDURE

Materials and Methods—The myoglobin that was used as the starting material for the chromatographic experiments was prepared from skeletal muscle by a modification of the methods of Drabkin (3) and Ginger, Wilson, and Schweigert (4) as described previously (5). The muscle was obtained at autopsy, within 4 to 14 hours of death, from patients who died of nonmuscular disorders and was stored at -5°C until used. Crystalline myoglobin was prepared by the method of Lugninhul (6). Spectral analysis showed that the myoglobin in these preparations was in the ferri- or met-form.

Dilute solutions of myoglobin were concentrated by one of three means. Saturation of aqueous solutions with ammonium sulfate at pH 7 completely precipitates myoglobin, which then can be collected by gravity filtration on filtered filter paper, suspended in a small amount of water, and dialyzed until free of ammonium sulfate. Concentration also was obtained by centrifugation at 96,000 \(\times\) g in the Spinco model L ultracentrifuge at 4°C for 72 hours. Under these conditions, myoglobin sediments to the bottom of the centrifuge tube and can be removed from the tube with a needle and syringe. The most effective means for concentrating myoglobin from very dilute solutions (<0.2%) was by adsorption on DEAE-cellulose. Myoglobin is adsorbed quantitatively from solutions <0.001 M in salt concentration, pH 8.9, at 6°C on DEAE-cellulose that has been equilibrated with 0.001 M Tris buffer, pH 8.9. Adsorption is most effective when the cellulose is packed in columns with dimensions of approximately 1 x 10 cm. The myoglobin is eluted from the cellulose with 1 M Tris buffer, pH 7.85, or 1 M sodium chloride (unbuffered). Buffer salts or sodium chloride are removed by dialysis.

The concentration of myoglobin was measured spectrophotometrically. The extinction coefficient \(E_{1%}\) at 414 m\(\mu\) of human myoglobin was determined for several preparations from simultaneous dry-weight determinations at 105°C and spectrophotometric analysis at 280 m\(\mu\). For a molecular weight of 17,500, the molar extinction coefficient is 3.4 x 10^4 between pH 5 and 9.

Zone electrophoresis on starch gels was performed by the method of Smithies (7). Starch gels were stained with Amido Black B (7) and with benzidine hydrochloride (8). Two-dimensional paper electrophoresis-chromatography of tryptic peptides was performed as described earlier (9). Spectral analyses were made with a Carey Model 14 automatic recording spectrophotometer. Amino terminal end group analyses were performed by the phenyl isothiocyanate and the fluorodinitrobenzene techniques (10). Carboxyl-terminal end group analysis was made with carboxypeptidase A. Amino acid analyses were performed with the Spinco model MS automatic amino acid analyzer (11, 12). Iron was estimated by the method of Gubler et al. (13). Carboxypeptidase A ( Worthington Biochemical Corporation, 3 X crystallized) was treated with diisopropyl fluorophosphate before use. Crystalline trypsin was a commercial preparation (Worthington).

Chromatography of Myoglobin—Myoglobin was chromatographed at 6°C on DEAE-cellulose that had been equilibrated with 0.005 M Tris buffer, pH 7.85. Jacketed columns (0.9 x 12 cm) which were cooled by circulation of water at 6°C were used for analysis of small (5 to 15 mg) amounts of myoglobin. Chromatography on a preparative scale (0.5 to 1.2 g) was achieved in the same manner with 5 x 15-cm jacketed columns. Typical results of a chromatographic separation of myoglobin performed on preparative columns are shown in Fig. 1. Four fractions \(F_1, F_2, F_3, F_4\) were eluted with the dilute Tris buffer, whereas

1 We would like to thank Mr. Boyd Lythgoe for his valuable assistance in performing these analyses.
one (F3) was eluted with 1 M Tris buffer, pH 7.8, at 25°. Fractions F1, F2, and F3 contained the majority of the heme protein. Fraction A, which emerged from the column at the hold-up volume, contained only none-heme protein. The yield of each fraction varied among different preparations; however, F1, F2, and F3 generally accounted for 55 to 65%, 15 to 20%, and 15 to 30%, respectively, of the total myoglobin applied to the column. It is noteworthy that the fractions had visibly different colors: F1 was brown, F3 was dark red, F3 was reddish-brown, and F4 was greenish-brown. Crystalline myoglobin gave the same chromatographic pattern as the noncrystalline myoglobin shown in Fig. 1.

When F1 and F2 were rechromatographed separately under the same conditions, two components were observed. Eighty per cent of F1 emerged from the column in the volume expected from its behavior as shown in Fig. 1, whereas the remainder behaved like F2. Fraction 2 on the other hand revealed approximately 25% of F1 on rechromatography. Because the rechromatographed samples could not be contaminated with another to this extent, these results suggest that the myoglobin in each fraction can be converted into the other chromatographic form. When F1 and F2 were chromatographed as oxymyoglobin or cyanometmyoglobin, they could not be distinguished from one another. This is seen in Fig. 2A (F1 and F2 oxymyoglobins) and in Fig. 2B (F1 and F2 cyanometmyoglobins). The small differences in the point of emergence probably reflect variations in the size of the columns used in the experiments. When unfractinated preparations were chromatographed as oxymyoglobin or cyanometmyoglobin, only one peak was observed in addition to F1.

Fraction 3 could not be purified further under the conditions described in Fig. 1. The most successful means for separating the myoglobins in this fraction was by chromatography as shown in Fig. 3. A gradient of sodium chloride formed with the cone-sphere arrangement (1850 ml, total volume) described by Sober and Peterson (14) was used in conjunction with a DEAE-cellulose column and 0.005 M Tris, pH 7.85, as the starting buffer. Six distinct fractions were obtained, only three of which were present in significant amounts (F1B, F2C and F3D). Because each component except F3 had a smaller (415/280 mp) absorption ratio than F1 and F2, it is likely that significant amounts of non-heme proteins contaminated all fractions but F3. Attempts to purify the other heme proteins in F3 by varying the chromatographic conditions were unsuccessful.

Electrophoresis of Myoglobin on Starch Gels—Zone electrophoresis on starch gels proved to be a useful means of characterizing unfractionated myoglobin. Fig. 4 shows the electrophoretic behavior of unfractionated myoglobin and of the chromatographically purified fractions. Three heme proteins could be distinguished. Occasionally a fourth, which migrated more rapidly than the others, was observed. Each of the three major heme proteins in unfractionated myoglobin was similar to one of the major chromatographic fractions (F1, F2, and F3). The heme protein that moves more rapidly than the major components in unfractusated preparations was found in F3 and presumably represents one of the minor components of this fraction (Fig. 3). Although it is evident that F1 shows a considerable amount of F2 and F2 shows significant amounts of F3, this result is analogous to the behavior of these fractions on rechromatography. Fraction B could not be distinguished from F3 by this method. It is noteworthy that F3 has a greater electrophoretic mobility than any of the other fractions. When unfractinated myoglobin...
FIG. 3. Rechromatography of myoglobin $F_1$. $F_2$, 60 mg, which was obtained as shown in Fig. 1, was converted to cyanometryglobin as described in Fig. 2B. The myoglobin was then applied to a 1 x 10 cm column of DEAE-cellulose which had been equilibrated with 0.005 m Tris buffer, pH 7.8, containing 0.001 m potassium cyanide at 6°. After the sample was absorbed at the top of the column, the column was developed at 6° by gradient elution as described in the text. Fractions of 5 ml were collected at a flow rate of 20 ml per hour.

FIG. 4. Zone electrophoresis of myoglobin on starch gels. Unfractionated myoglobin and the chromatographic components derived from myoglobin were analyzed at pH 8.6 by the methods of Smithies (7). Electrophoresis was performed for 18 hours with a potential gradient of 4 volts per cm. After longitudinal cuts of these gels were made, the proteins were detected by staining with Amido Black B. When cyanometmyoglobins were analyzed, potassium cyanide 0.001 m was added to the buffer. In the top half of the figure, metmyoglobins are shown. The lower half, showing cyanometmyoglobins, illustrates the fact that electrophoretic differences between $F_1$ and $F_2$ disappear when the cyanometmyoglobins are used.

Comparison of Tryptic Peptides from Chromatographically Purified Myoglobins—In order to determine whether the chromatographically pure myoglobins differed from one another in primary structure, tryptic digests of each myoglobin were analyzed on paper by a two-dimensional electrophoretic-chromatographic technique (9). Myoglobin, 25 mg, dissolved in 2 to 3 ml of water, were treated at -10° with 25 volumes of an acetone-HCl mixture according to the methods of Rossi-Fanelli and Antonini (15). The resulting globin was dissolved in 2.5 ml of water, and the pH of the solution was adjusted to 8.0. Trypsin, 0.5 mg, dissolved in 0.1 ml of 0.001 m HCl, was added to start
FIG. 5. Two-dimensional peptide patterns of the tryptic peptides from human myoglobins F1 and F2. An aliquot of the tryptic digest which contained the peptides from 1 to 2 mg of myoglobin was resolved electrophoretically on Whatman 3MM paper for 3 hours at 10 volts per cm in pyridine acetate buffer, pH 6.5. The peptides then were resolved chromatographically in the second dimension for 20 hours with butanol-acetic acid-water (200:30:75). Peptides were located with a ninhydrin spray reagent.

the digestion and the mixture was incubated at 40°. Throughout the digestion, the pH was maintained between pH 7.8 and 8.2 by the addition of 0.1 N NaOH. The precipitate of globin disappeared during the first 15 to 30 minutes and hydrolysis was complete after 90 minutes. No differences were observed in the rates of hydrolysis of the different myoglobin fractions. Analysis of aliquots of the tryptic digests obtained in this manner gave peptide patterns like those shown in Fig. 5. Fractions 1, 2, and B were indistinguishable by this method of analysis. At least 24 distinct peptides were found, a number close to that expected from the lysine and arginine content of human myoglobin (Table I). Fractions 1, 2, and B also gave identical patterns when the papers were sprayed with reagents which specifically detect histidine (16), tryptophan (17), arginine (18), tyrosine (19) and sulfur-containing peptides (20). Fraction 3D (Fig. 3), which was the only myoglobin from Fraction 3 that was not contaminated with non-heme protein, gave patterns that differed from those in Fig. 5. Several spots in addition to those peptides found in Fractions 1, 2, and B were observed. This result, together with the difference in electrophoretic mobility noted when F1 and F2 were compared by zone electrophoresis, suggests that the primary structure of the myoglobin in F1 may differ from that of F1, F2, and F3. However, since F3 may contain small amounts of non-heme protein, no definite conclusions about its primary structure can be drawn at this time. Additional experiments will be necessary to clarify the nature of this myoglobin.

PHYSICAL CHARACTERIZATION

Spectral Analysis—The ultraviolet and visible spectra of each of the myoglobin components were determined. The wave lengths of maximal absorption for four of the chromatographically purified myoglobins are listed in Table II. F1 has absorption characteristics of acid metmyoglobin, whereas F2 and F3 have those of alkaline metmyoglobin (21). A portion of the spectra obtained with these types of metmyoglobin is shown in Fig. 6. The spectra of F1 and F2 were identical when they were converted to either the reduced, oxy-, cyanmet-, or carbamyl-forms.

Sedimentation and Diffusion Studies—These analyses were made in 0.1 M Veronal buffer, pH 8.5, with the Spinco model E ultracentrifuge; each run was made at 59,780 r.p.m., 20°. The photographic plates were read with a microcomparator. All other operations were the same as reported in earlier studies from this laboratory (22, 23).

F1 and F2 sedimented as single, homogenous peaks. Their rate of sedimentation was a function of the protein concentration. Although the red color of myoglobin at concentrations greater than 6 mg per ml prevented more extensive studies, the S20,w, value at infinite dilution was calculated from the data available to be 1.815S.

Diffusion studies were performed in the Spinco model H electrophoresis-diffusion apparatus with the aid of the Rayleigh interference optical system. Diffusion constants were calculated by the methods of Longsworth (24) and Schachmann (25). F1 was analyzed in 0.1 M Veronal buffer, pH 8.5, at a protein concentration of 1.1 mg per ml. The color of the myoglobin prevented measurements at higher concentrations. Photographs of the interference patterns were calculated between 20 and 56 hours after formation of the boundary. The patterns obtained were read on a microcomparator. The results were corrected for viscosity, density, and temperature in the usual
TABLE I

Amino acid composition of human myoglobin

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid residue per 100 g of protein</th>
<th>Amino acid residue per 100 g of protein</th>
<th>N as per cent of total N</th>
<th>Calculated no. of residues</th>
<th>Assumed no. of residues</th>
<th>No. of residues (Ref. 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>5.71</td>
<td>4.56</td>
<td>5.22</td>
<td>11.2</td>
<td>11</td>
<td>11</td>
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<tr>
<td>Arginine</td>
<td>2.65</td>
<td>2.38</td>
<td>5.10</td>
<td>2.7</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Aspartic acid</td>
<td>9.21</td>
<td>7.97</td>
<td>5.80</td>
<td>12.1</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>17.31</td>
<td>15.20</td>
<td>9.87</td>
<td>20.6</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Glycine</td>
<td>12.5</td>
<td>10.75</td>
<td>7.08</td>
<td>14.6</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Histidine</td>
<td>8.21</td>
<td>7.26</td>
<td>3.31</td>
<td>9.3</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.03</td>
<td>4.34</td>
<td>3.21</td>
<td>6.7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Leucine</td>
<td>12.19</td>
<td>10.52</td>
<td>7.00</td>
<td>16.3</td>
<td>16</td>
<td>18</td>
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<tr>
<td>Lysine</td>
<td>16.12</td>
<td>14.14</td>
<td>18.50</td>
<td>19.3</td>
<td>19</td>
<td>22</td>
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<tr>
<td>Methionine</td>
<td>2.45</td>
<td>2.16</td>
<td>1.38</td>
<td>2.9</td>
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<td>3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.22</td>
<td>5.54</td>
<td>3.16</td>
<td>6.6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Proline</td>
<td>3.99</td>
<td>3.37</td>
<td>2.94</td>
<td>6.1</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Serine</td>
<td>4.58*</td>
<td>3.77</td>
<td>3.05</td>
<td>7.6</td>
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<td>7</td>
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<tr>
<td>Threonine</td>
<td>2.94*</td>
<td>2.50</td>
<td>2.67</td>
<td>4.3</td>
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<td>4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.60†</td>
<td>3.28</td>
<td>2.96</td>
<td>3.0</td>
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<tr>
<td>Tyrosine</td>
<td>2.35</td>
<td>2.12</td>
<td>1.09</td>
<td>2.3</td>
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<tr>
<td>Valine</td>
<td>5.26</td>
<td>4.45</td>
<td>3.77</td>
<td>7.8</td>
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<td>6</td>
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<tr>
<td>Ammonia</td>
<td>1.08‡</td>
<td>5.66</td>
<td>11.1</td>
<td>11</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Glutamine</td>
<td></td>
<td></td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Asparagine</td>
<td></td>
<td></td>
<td>71†</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
</tbody>
</table>

| Total      | 114.04                                 | 98.31                                  | 102.45                  | 154                        | 154                     | 150                       |

* These values obtained by extrapolation to zero time hydrolysis.
† These values obtained from analysis of complete enzymic hydrolysates.
‡ These values omitted from the total.

The molecular weight of myoglobin, computed from the sedimentation and diffusion constants, is 17,900. The partial specific volume of 0.43, which was used in calculating the molecular weight, was estimated from the amino acid composition reported in Table I.

CHEMICAL CHARACTERIZATION

Amino Terminal End-group Analysis—Globin, prepared from F1 and F2, gave glycine as the only detectable end group. Hydrochloric acid, 0.05 ml, in 5 ml of ethanol was added to 2 ml of 1% sodium bicarbonate containing 25 mg of globin. After the mixture had reacted for 2 hours at 25°C, the insoluble dinitrophenylglobin was collected by centrifugation, washed three times successively with water, ethanol, and ether, and then air-dried for 5 days. Samples of the dinitrophenylglobin were hydrolyzed with the formic acid-acetic anhydride-perchloric acid mixture of Hanes, Hird, and Isherwood (26) for 2 hours at 100°C. Chromatographic analysis of hydrolysates (10) revealed only dinitrophenylglycine in an amount corresponding to 0.73 moles per mole of dinitrophenylglobin (mol. wt. 17,500). Because this value was not corrected for the weight of the dinitrophenyl

Similar results were obtained when globin was analyzed on paper strips (1 × 10 cm) with the phenyl isothiocyanate method described by Shelton and Schroeder (27). Only glycine phenyl isothiocyanate was detected on Solvents A, D, and F of Sjöquist (28) and Edman and Sjöquist (29). Although no quantitative estimation was made of the amount of this derivative, the absence of other detectable phenyl thiocyanates precludes the possibility of other amino end groups.

Carboxyl Terminal End-group Analysis—Globin is not sufficiently soluble at pH 8 to allow uniform digestion with carboxypeptidase A. Digestion was achieved, however, under conditions similar to those used by Guidotti for the α- and β-chains of human hemoglobin (30). Globin, 25 mg, dissolved in 2.5 ml of water was mixed with 0.125 ml of 0.2 M sodium lauryl sulfate and the pH of the mixture was adjusted to 8. After addition of 0.6 mg of carboxypeptidase A (0.05 ml) the mixture was incubated at 40°C. Aliquots, 0.7 ml, were removed at 15, 30, and 55 minutes after addition of the enzyme, and acidified to stop hydrolysis. The amino acid content of each aliquot was measured by the methods of Spackman, Stein, and Moore (11, 12). Fig. 7 shows the rate of release of the amino acids. Although it is impossible to distinguish between glutamic, asparaginic, and aspartic on the columns used for analysis, only glutamine was seen on paper chromatograms at 15, 30, and 55 minutes. It is evident that the

![Fig. 6. The absorption spectra of myoglobin Fractions 1 and 2.](http://www.jbc.org/)

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Amino acids are released in the order glycine > glutamine > phenylalanine > leucine, results which suggest a carboxyl terminal sequence similar to that found on analysis of horse myoglobin by similar methods (31, 32), namely, Leu-Phe-Glu-Gly. However, it is apparent that more than one residue of glycine is released, which suggests that this amino acid occurs in more than one position in the carboxyl terminal sequence. Indeed, unpublished studies,2 have shown that a peptide from a tryptic digest of globin possesses the sequence Glu-Leu-Gly-

The composition of human myoglobin estimated by these methods is given in Table II. The weight recovery of amino acid residues and the nitrogen recovery, which are not corrected for the heme content of myoglobin, are within the precision of the analytical methods employed. The number of residues was calculated from the minimal molecular weight that was obtained with each of the amino acids on the basis of a molecular weight of 17,450. The molecular weight computed from the assumed number of residues is 17,330. When corrected for the heme content of myoglobin, a molecular weight of 17,620 is obtained, in close agreement with the value 17,900 estimated from sedimentation-diffusion measurements, and the value 17,450 calculated from the iron content.

It is noteworthy that the composition obtained by these methods differs somewhat from that reported earlier by Rossi-Fanelli, Cavallini, and DeMarco (33). The improved methods of chromatographic analysis of amino acids that were used here were not available when the earlier analyses were made, nor was a chromatographically purified myoglobin preparation examined at that time. These factors may account for the small differences observed. One analysis of a hydrolysate of F2 (see above) gave essentially the same composition shown in Table I for F1. F2 was not analyzed since its composition would be altered by the non-heme proteins that are present in this material.

DISCUSSION

It is evident that the various components in human metmyoglobin which can be distinguished by electrophoretic or chromatographic means differ structurally either in their heme prosthetic group or in their globin moiety. The two major components, F1 and F3, appear to differ only in their prosthetic group. F1 has the spectral properties of acid metmyoglobin and F3 of alkaline metmyoglobin. These forms are in equilibrium with one another as shown schematically by the following equation, where MbFe+-OH2 represents acid metmyoglobin and MeFe(OH) represents alkaline metmyoglobin.

\[
\text{MbFe}^+ - \text{OH}_2 \rightleftharpoons \text{MeFe} - \text{OH} + \text{H}^+
\]

The pKₐ of this reaction for horse myoglobin at 20° in the low salt concentration used in this study is approximately 8.9 (36). Because the acid and alkaline species are in equilibrium, and the amount of each depends on the pH of the solution, it is striking that one species can be isolated from the other by chromatographic or electrophoretic means. In the chromatographic experiments that employ Tris buffers at pH 7.8 to 7.9, approximately 85% of the myoglobin (excluding F3) is in the acid form, the remainder in the alkaline form. These are close to the amounts expected at this pH if the pK of the reaction for human myoglobin is close to that of horse myoglobin.

F3, a chromatographic component which is present in minor amounts, appears to possess the same globin moiety as F1 and F2. Although its electrophoretic mobility on starch gels is unaffected by reaction with cyanide, other observations suggest that it has an altered prosthetic group. It possesses a distinct brown-green color, absorbs slightly at 620 mp, and has a lower (415/280 mp) absorption ratio than F1 or F2. Only small amounts of this material were available and an exact spectral characterization was impossible. However, the color and spectral properties of F3 suggest that it contains a modified protoporphyrin ring system similar to cheloglobin or verdoglobin (20). This form of the protoporphyrin ring probably would not be expected to exist in vivo but could be formed either after death before the muscle is removed and frozen, or during the preparative procedure.
In contrast to \( F_1 \), \( F_2 \), and \( F_3 \), \( F_2 \) appears to contain more than one myoglobin component as well as non-heme proteins. When \( F_3 \) is analyzed electrophoretically on starch gels (Fig. 4), it migrates at pH 8.6 faster than either \( F_1 \) or \( F_2 \). As isolated from DEAE-cellulose (Fig. 1), it has a spectrum similar to that of alkaline metmyoglobin. Although it readily reacts with cyanide, as well as with oxygen or carbon monoxide after reduction with dithionate, its electrophoretic mobility is unaffected. Redetermination, with the use of a salt gradient with DEAE-cellulose (Fig. 3) at pH 7.8, partially resolves \( F_3 \) into six different components. Despite these differences in the properties of \( F_3 \), the presence of small quantities of contaminating protein in \( F_2 \), the only \( F_2 \) component with a 415/280 nm ratio similar to \( F_1 \) or \( F_3 \), prevent definite conclusions to be made about primary structural differences.

It is interesting to compare the chromatographic behavior of human myoglobin with the behavior of myoglobin from other species. Brown (37) reported recently the chromatographic analysis of whale, bovine, and tuna myoglobins on DEAE-cellulose under conditions similar to those developed here. At pH 8.2 to 8.6 in 0.005 to 0.05 M Tris buffer, the bovine and whale myoglobins chromatographed as single, apparently homogeneous proteins. Bluefin tuna myoglobin, however, shows three peaks, each of which is analogous both in point of emergence and in amount to one of the three major peaks in human myoglobin. Lack of detailed studies of the three tuna components prevents more detailed comparison. Chromatographic characterization of the myoglobin from other species has been achieved with carboxylic acid ion-exchange resins or celluloses. Rumen (38) has shown that five distinct components can be resolved from whale myoglobin at pH 8.6, all of which are reported to possess the same spectral properties. From the limited data presented it is impossible to judge whether any of these components correspond to acid or alkaline metmyoglobins. Edmundson and Hirs (39), with Amberlite IRC-50 at pH 5.86, also found five components in whale myoglobin. Because only acid metmyoglobin would be present at this pH, the heterogeneity of whale myoglobin appears to result at least in part from primary structural differences (39). Similarly, Åkesson and Theorell (40) have shown that equine myoglobin can be resolved into at least three components at pH 6.9 on carboxymethyl cellulose. This heterogeneity also appears to result from primary structural differences.

**Table III**

<table>
<thead>
<tr>
<th>Species</th>
<th>Amino terminal sequence</th>
<th>Carboxyl terminal sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human...</td>
<td>Gly-Leu-Ser-</td>
<td>-Leu-Gly-Phe-Gly-Glu-NH₂</td>
<td>This paper*</td>
</tr>
<tr>
<td>Whale...</td>
<td>Val-Ala-Gly-</td>
<td>-Leu-Gly-Tyr-Gly-Glu-NH₂</td>
<td>(40)</td>
</tr>
<tr>
<td>Horse...</td>
<td>Gly-Leu-</td>
<td>-Leu-Asp-Phe-Gly-</td>
<td>(30, 33)</td>
</tr>
<tr>
<td>Seal...</td>
<td>Gly-</td>
<td></td>
<td>(38)</td>
</tr>
</tbody>
</table>

* Detailed sequence analysis of human myoglobin will be published later.

Although the properties of human myoglobin that have been determined in this study are similar to those of myoglobins from other species, certain structural differences between human myoglobin and other myoglobins are evident. The amino terminal and carboxyl terminal sequences of four species are shown in Table III. Certain species variations occur although none of these would be predicted to alter markedly properties of the myoglobin. The tryptic peptide patterns show distinct differences from those of other myoglobins that have been analyzed. Stockell (41) has compared the peptide patterns of seven species (5 mammals, 1 bird, and 1 reptile) and found considerable similarity in certain unique peptides. The methods employed here for the preparation of peptide patterns differ from those used by Stockell and direct comparison is impossible. The patterns of whale* and human myoglobin prepared by our methods are shown in Fig. 8. Several similarities are evident among the peptides. On the basis of specific color reactions which certain peptides give for histidine, tryptophan, arginine, or tyrosine, at least eight peptides would seem to correspond to one another in the two myoglobins. The similar peptides are given identical numbers in the figure. The other peptides cannot be related easily to one another although the fact that human myoglobin does not yield an insoluble residue after tryptic digestion accounts for the larger number of peptides seen on the human pattern. Whereas these results are not unexpected, it is surprising that the amino acid composition of whale (39) and human myoglobin differs by at least 35 residues of the approximately 153 residues in each molecule.

**SUMMARY**

1. Human metmyoglobin can be resolved chromatographically on DEAE-cellulose into at least four distinct heme protein components.
2. The two chromatographic components that account for 75 to 80% of the total myoglobin of normal human muscle appear to differ from one another only in their heme prosthetic group.
3. Some of the chromatographic components present in minor amounts in human myoglobin appear to differ in their primary structures. The exact differences have not been established at this time.
4. The physical and chemical properties of chromatographi-

* Whale myoglobin (crystalline) was kindly supplied by Dr. John Kendrew.
ally pure human myoglobin are similar to those reported previously.

5. Certain primary structural differences between human globin and the globin from other species have been shown.

REFERENCES

The Characterization of Adult Human Myoglobin
Gerald T. Perkoff, Robert L. Hill, Douglas M. Brown and Frank H. Tyler


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