OXIDATION-REDUCTION POTENTIALS OF THE METMYOGLOBIN-MYOGLOBIN SYSTEM*

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The similarity in composition and function between myoglobin and hemoglobin has prompted numerous comparisons of the physicochemical properties of these two important biological pigments (8). Quantitative differences in their molecular weights (12, 14, 18), solubilities (9), absorption spectra (19), and equilibria with oxygen or carbon monoxide (6, 20) have been established. The presence in both proteins of ferroprotoporphyrin (11, 13), which can be reversibly oxidized (1, 4, 16), suggests the comparison of their oxidation-reduction potentials. The oxidation-reduction behavior of the hemoglobin system has been described previously (15, 16).

It is the purpose of this paper to report oxidation-reduction titrations of myoglobin performed at various pH values between 5.9 and 7.4 with the aid of electromotively active mediators. The oxidation-reduction reaction is described by the electrode Equation 1 with n = 1.

\[ E_h = E_m + \frac{RT}{nF} \ln \frac{[\text{metmyoglobin (Fe}^{+++})]}{[\text{myoglobin (Fe}^{++})]} \]  

**EXPERIMENTAL**

Crystalline metmyoglobin was prepared from horse hearts according to Morgan’s modification of Theorell’s procedure (9, 17).1 After the metmyoglobin was crystallized by saturation with ammonium sulfate, the crystals were concentrated by the centrifuge, and ammonium sulfate was removed by dialysis against distilled water. Methemoglobin was removed from the preparation as suggested by Morgan (9), by rotating a cellophane bag containing the myoglobin solution in 3 M phosphate buffer having a

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* The “Studies on myoglobin” (9), begun by Dr. Vincent E. Morgan in 1934, were interrupted in 1939 by his untimely death. Through arrangement with Professor D. Bruce Dill, unpublished data of Dr. Morgan on the titration of myoglobin in the absence of electroactive mediators were made available for publication in this paper. A portion of Dr. Morgan’s data is contained in his dissertation, entitled “Some aspects of the physical chemistry of horse heart carboxymyoglobin,” Harvard University, Cambridge, 1937. (J. F. T.)

1 These hearts were obtained through the courtesy of the Massachusetts Antitoxin and Vaccine Laboratory.
pH of 6.6. This procedure had the advantage of keeping the solutions concentrated. An appreciable quantity of brown crystalline material, presumed from its solubility (9) and crystalline form (7) to be methemoglobin, was then removed by filtration. Phosphate was removed by dialysis against distilled water, yielding stock solutions containing metmyoglobin in a concentration of approximately 2 per cent. These were used directly in the preparations of solutions to be titrated.

Spectroscopic observation indicated that the myoglobin was converted to metmyoglobin during the course of the preparation. A small amount of ferricyanide was added, before the reductive titrations were commenced, to insure complete oxidation of the myoglobin. The concentration of metmyoglobin in the stock solutions was estimated by spectrophotometric observations at a wave-length of 630 m\(\mu\), in phosphate buffer at pH 7.0, the extinction coefficient determined by Theorell (19) being used.

In order to study the oxidation-reduction potentials from pH 5.9 to 7.4, stock phosphate buffers were prepared from the tables of Green (5), but of 5 times the concentration necessary to give, on dilution, a final ionic strength of 0.2. The stock buffers were diluted to 5-fold their original volume, either with the stock metmyoglobin solutions or with water, to provide the solutions used for the oxidation-reduction titrations and pH measurements. The pH values of the myoglobin-buffer mixtures were measured at the end of each titration with the use of the glass electrode previously described (15). The electrode potentials and pH values have been referred to the normal hydrogen electrode after the conventions of Clark (3). (This corresponds to assigning the value 4.62 to the pH of standard acetate, at 30\(^\circ\)C.)

Metmyoglobin solutions prepared as described above were titrated with reduced anthraquinone-\(\beta\)-sulfonate by the technique and with the apparatus previously described (16). Tolyylene blue and cresyl blue were used as mediators. Except at the ends of the curves, stable potentials without drifting were obtained within less than 10 minutes, and over fifteen points were obtained in the course of each titration, thus permitting accurate characterization of the curves.

In early experiments performed by one of us (V. E. M.), the oxidation-reduction potentials of metmyoglobin-myoglobin mixtures in the absence of mediators were found to exhibit the sluggish behavior characteristic of hemoglobin systems (16). It was necessary under these conditions to wait from 1 to 2 hours after each addition of oxidant or reductant for the establishment of even apparent equilibrium. This precluded the possibility of obtaining more than a few points in any one titration. In many instances lack of agreement between electrodes, and drifting potentials, contributed to the uncertainty of the data.
Results

Table I summarizes the results of a titration in the presence of mediators at pH 6.95. The values for $E'_m$ given in the sixth column have been calculated for a transfer of 1 electron during the titration according to Equation 1. The agreement is seen to be satisfactory. Analysis of all titration curves in the presence of mediators, from pH 5.9 to 7.4, by the objective method of Reed and Berkson (4, 10) has shown that 1 electron is involved.

The solution reduced at pH 6.95 was subsequently reoxidized by titration with ferricyanide, yielding data in entire agreement with those given in Table I for the reduction.
Table II summarizes the results of five titrations performed upon myoglobin in the presence of mediators, in phosphate buffer solutions from pH 5.9 to 7.4. $E'_m$, the oxidation-reduction potential of an equimolar mixture of metmyoglobin and myoglobin, is plotted against pH in Fig. 1. There are also presented, for reference, the values of $E'_m$ at various pH values previously obtained by the titration of the hemoglobin-methemoglobin system, both in the presence and absence of urea (15, 16).

The values of $E'_m$ from six titrations, carried out in the absence of mediators, agreed approximately with the titrations carried out with mediators present. These data have been included in Fig. 1. Other titrations yielded values of $E'_m$ which scattered widely and were obviously aberrant and irreproducible. Especially at higher pH values the potentials were much more negative than the results shown in Fig. 1, approaching the

**Table II**

**Metmyoglobin-Myoglobin, Relation of $E'_m$ to pH at 30°**

Phosphate buffers, ionic strength = 0.20; myoglobin concentration 1.0 to 1.1 $\times$ 10$^{-3}$ M; toluylene blue 2.5 $\times$ 10$^{-6}$ M; cresyl blue 2.5 $\times$ 10$^{-6}$ M.

<table>
<thead>
<tr>
<th>pH</th>
<th>$E'_m$ (vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.93</td>
<td>+0.0537</td>
</tr>
<tr>
<td>6.40</td>
<td>+0.0508</td>
</tr>
<tr>
<td>6.95</td>
<td>+0.0460*</td>
</tr>
<tr>
<td>6.95</td>
<td>+0.0460†</td>
</tr>
<tr>
<td>7.39</td>
<td>+0.0433</td>
</tr>
</tbody>
</table>

* Reported in detail in Table I.
† Reoxidation with K$_3$Fe(CN)$_6$.

**Fig. 1.** Relation of $E'_m$ to pH. $E'_m$ is the potential of the system at 50 per cent reduction. Curve A, myoglobin, ○ experimental data obtained with the aid of mediators (Table II), solid line drawn free-hand, □ experimental data obtained without using mediators; Curve B, hemoglobin in 4 M urea solution (15); Curve C, hemoglobin (15, 16).
values reported for globin hemochromogen (1). The single value reported by Bechtold and Pfeilsticker (2), $-0.08$ volt at pH 7.4, is also in the same negative range.

DISCUSSION

The line connecting the values of $E'_m$ for the metmyoglobin-myoglobin system in the presence of mediators (Fig. 1) has a slope of about $-0.007$ volt per pH unit. Our data do not cover a sufficiently wide pH range to permit the interpretation of the change in $E'_m$ with pH in terms of the acid-base dissociation constants involved.

The observation that $n$ equals unity in the oxidation-reduction reaction of myoglobin is consistent with a transfer of 1 electron per molecule containing one heme group, thus contributing additional evidence that the molecular weight of myoglobin is 17,000.

The oxidation-reduction potential of the metmyoglobin-myoglobin system is much more negative than that of the methemoglobin-hemoglobin system. For example, at pH 7, the value of $E'_m$ for the myoglobin system is $+0.046$; for the hemoglobin system, it is $+0.144$ (15, 16). Although the oxidation-reduction level of the intracellular environment of myoglobin is probably sufficiently low under ordinary circumstances to prevent the formation of metmyoglobin, it is probable that the intracellular presence of materials capable of converting hemoglobin to methemoglobin would be even more effective in converting myoglobin to metmyoglobin.

SUMMARY

1 electron is transferred in the reduction of metmyoglobin to myoglobin. The oxidation-reduction potential becomes only slightly more negative as the pH is increased from 5.9 to 7.4.

The oxidation-reduction potential of an equimolar mixture of metmyoglobin and myoglobin is $+0.046$ volt at pH 7.0 and 30°.

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