NOTES ON MYOGLOBIN PREPARATION AND IRON CONTENT

BY WILLIAM J. BOWEN

(From the Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland)

(Received for publication, June 22, 1948)

Since the first description by Theorell (11) of a process of isolating and purifying myoglobin from horse heart by crystallization, several workers have succeeded in preparing myoglobin by Theorell's procedure. Roche and Vieil (8) and Rossi and Aragona (9) have described methods less arduous than Theorell's. Their methods, however, sacrifice yield to gain simplicity. We have developed a modification of Theorell's procedure for preparing horse heart myoglobin in which the more than twenty steps described by him are reduced to about ten with no sacrifice of yield. During the work, difficulty was experienced in attaining purity of the preparations based on the value for the iron content, 0.345 per cent, reported by Theorell (11) and others. It became apparent that the value 0.345 per cent iron is greater than the value computed from the ratio in myoglobin of iron content (1 atom per molecule) and the molecular weight. The results of analyses of our preparations indicate that the value computed from the molecular weight derived from sedimentation and diffusion constants can be used as a criterion of purity of myoglobin.

Preparation

At a slaughter-house, horse hearts are removed as soon as possible and perfused through the coronary arteries with tepid 0.85 per cent NaCl solution (made on the site with warm tap water) until the solution emerging from the veins is colorless. About 15 liters of solution are used per heart. The auricles and as much fat and connective tissue as practical are trimmed away from the ventricles. In the laboratory the work is carried out at 1–5° as far as possible. The ventricular flesh is ground by an electrically driven meat chopper, and the ground meat is mixed with a chilled volume of water equal in ml. to the weight of the meat in gm. The mixture is stirred well and left in the cold overnight.

The following morning the extract is separated by centrifuging at 1000g or more. The extract is then partially purified by making the solution 3 M (50 per cent of saturation) with ammonium sulfate and separating the precipitate thus salted out by either centrifugation (1000g) or filtration. The supernatant solution is put into cellophane sausage bags 3 or 4 inches
in diameter, which are then immersed in a chilled bath of saturated ammonium sulfate that contains an excess of the salt and is adjusted to pH 7.1 to 7.3 with NH₄OH. The solution outside the bag is stirred with an electric stirrer for 24 hours.

Crystals of myoglobin appear in a day or so gathered in typical clusters (11), the form of which resembles sheaves of grain. The clusters increase in size for 2 or 3 weeks and reach lengths up to 1 ml. The myoglobin can be left in the baths indefinitely at about 5°. (We have stored it thus for 1 year.) To isolate the crystals from the amorphous impurities, the contents of the dialysis bag are centrifuged at 1000g for 10 minutes. The supernatant solution with as much of the amorphous material as possible is aspirated from the layer of crystals. The remaining precipitate of crystals and amorphous matter is resuspended in saturated ammonium sulfate¹ and centrifuged at 200g to 300g for 5 minutes. The supernatant solution is again removed by aspiration. The process of resuspension, centrifugation, and aspiration is repeated three times. The precipitate of crystals is next suspended in saturated ammonium sulfate¹ in a 500 ml. graduated cylinder. After 24 hours the larger crystals have settled to the bottom and the solution above them is removed by aspiration. This process of suspension, settling, and aspiration in the cylinder is carried out six times.

After the final washing, the ammonium sulfate is diluted until the myoglobin is dissolved. The solution is then dialyzed in a cellophane bag, 0.75 to 1.0 inch in diameter, in the cold against running distilled water for 24 hours or until a portion of the extract gives no indication of sulfate when tested with 10 per cent barium chloride. The dialysis is carried out on a rocker arm with a bubble in the bag to assure mixing. When the solution is removed from the bag, it contains a light precipitate which is apparently of no consequence and is removed by filtration. The solution of myoglobin thus obtained can be stored indefinitely in a refrigerator if a drop of chloroform is added.

Iron Content

In his original publication, Theorell (11) presented four determinations of iron in horse heart myoglobin which averaged 0.345 per cent. Later, Theorell and de Duve (12) obtained 0.340 per cent in human myoglobin. Either of these values is sometimes claimed to be the iron content of hemoglobin (4, 5, 11), and since myoglobin, like hemoglobin, contains 1 iron atom per unit of heme, it has been accepted as the iron content of myoglobin. Millikan (5) gives 0.345 per cent in a review of research on myoglobin.

¹ In order to lessen the possibility of contaminating the myoglobin with iron contained as an impurity in ammonium sulfate, the saturated solution was filtered after standing several weeks and the iron in it had precipitated.
globin, and Rossi and Aragona (9) state that they obtained this proportion repeatedly. Drabkin (4) found 0.340 ± 0.002 per cent. When used to calculate the molecular weight of myoglobin, 0.345 per cent iron gives 16,186. The value 0.340 gives 16,424.

The molecular weight of horse heart myoglobin has been determined by five methods. These are listed in Table I, together with the calculated values for iron content on the assumption of 1 atom of iron per molecule. It is to be noted that the results are consistently lower than that obtained by Theorell. Zinoffsky (14) found, by painstaking methods, that the iron content of horse hemoglobin is also less than 0.345 per cent; he obtained 0.335 per cent. Valer (13), working with care equal to Zinoffsky's, obtained 0.330 per cent.

### Table I

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16,850</td>
<td>0.331</td>
<td>Osmotic pressure</td>
<td>Roche and Vieil (8)</td>
</tr>
<tr>
<td>17,200</td>
<td>0.325</td>
<td>Sedimentation and diffusion</td>
<td>Polson (6)</td>
</tr>
<tr>
<td>17,500</td>
<td>0.319</td>
<td>Sedimentation-equilibrium</td>
<td>&quot; (10)</td>
</tr>
<tr>
<td>17,534*</td>
<td>0.318</td>
<td>Bergmann and Niemann (1)</td>
<td>Roche and Derrien (7)</td>
</tr>
<tr>
<td>17,600</td>
<td>0.317</td>
<td>Rule of simple multiples</td>
<td>Pedersen (10)</td>
</tr>
</tbody>
</table>

* Roche and Derrien actually give 16,934, but they neglected to add the value of the molecular weight of 1 heme molecule (600) to the weight of the globin portion determined by them.

The molecular weight 17,500 (Table I), according to Pedersen (10), is the mean of four values obtained from determinations of the sedimentation equilibrium by Polson. Polson (6) also determined the molecular weight of myoglobin from the same preparation from sedimentation and diffusion constants, with correction for particle size. In this instance he obtained 17,200. Technical difficulties prevent the exact measurement of these values by sedimentation and diffusion methods and the publications cited give few of the data necessary for statistical evaluation. Polson (6) presents five experimental diffusion constants taken on two concentrations of myoglobin, which vary less than 1.1 per cent from the mean; however, according to Edsall (3), Svedberg has estimated the probable error in diffusion and sedimentation constants as 2 to 3 per cent and in calculated molecular weights as 5 to 10 per cent.

We have analyzed several preparations of myoglobin, prepared as described above, for iron. These analyses were made by the method described
by Delory (2), with \( \alpha, \alpha' \)-dipyridyl as an indicator. To establish the accuracy of the method, a statistical analysis of its reliability was made. First, twelve standards, to each of which were added 25 \( \gamma \) of iron per 10 ml., were analyzed. These gave an average iron content of 25.02 \( \gamma \) (\( \sigma = 0.80 \); coefficient of variation = 3.2 per cent). Secondly, nine samples of 1 ml. each from a single solution of horse heart myoglobin known to be only partially pure were analyzed for iron content and dry weight. The iron ranged from 6.00 to 6.19 \( \gamma \), with an average value of 6.10 (\( \sigma = 0.2 \); coefficient of variation = 3.3 per cent). The dry weights ranged from 1.97 to 2.10 mg. per ml. and averaged 2.03 mg. (\( \sigma = 0.14 \); coefficient of variation = 6 per cent).

Finally eight preparations of myoglobin of different concentrations were analyzed by this method. The determinations of iron in them were 0.313, 0.317, 0.305, 0.322, 0.352, 0.325, 0.340, and 0.306 per cent, average 0.323 (\( \sigma = 0.043 \); coefficient of variation = 13.3 per cent).

The possibility exists that this value for the iron content of myoglobin is affected by loss of either the heme or globin of denatured molecules. The solubility and stability of globin from myoglobin are unknown for the conditions of the preparation; however, from knowledge of the relative stabilities of globin and heme of hemoglobin, one would expect the heme to be more stable than the globin. If this obtains for myoglobin, denaturation would increase the iron content rather than decrease it, and would give values equal to or greater than 0.345 per cent.

The average value for iron content of myoglobin, 0.323 per cent, is in good agreement with the composition calculated from the molecular weights obtained by the sedimentation-diffusion studies (see Table I) discussed above. It agrees substantially with values for iron in horse hemoglobin found by Zinoffsky (14) and Valer (13). When used to calculate the molecular weight of myoglobin, 0.323 per cent gives 17,288. This result agrees well with Polson’s (6, 10) determination of the molecular weights of myoglobin, 17,200 and 17,500.

**SUMMARY**

A method to isolate and purify myoglobin of horse heart is described. The theoretical percentage composition with respect to iron in myoglobin is believed to be nearer 0.323 than the commonly accepted value 0.345. Eight preparations of myoglobin were analyzed. They were found to have values for iron ranging from 0.305 to 0.352 per cent, with an average of 0.323 per cent. When used to calculate the molecular weight of myoglobin, 0.323 per cent gives 17,300, which is in good agreement with the results of sedimentation and diffusion studies of myoglobin.
BIBLIOGRAPHY


NOTES ON MYOGLOBIN PREPARATION AND IRON CONTENT
William J. Bowen


Access the most updated version of this article at http://www.jbc.org/content/176/2/747.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/176/2/747.citation.full.html#ref-list-1