Reactivity of Ferrous Myoglobin at Low pH*

(Received for publication, June 23, 1977, and in revised form, September 9, 1977)

GIUSEPPE M. GIACOMETTI, T. G. TRAYLOR, PAOLO ASCENZI, MAURIZIO BRUNORI, AND ERALDO ANTONINI

From the Department of Chemistry, University of Rome, 00185 Rome, Italy and the Department of Chemistry, University of California, San Diego, La Jolla, California 92037

SUMMARY

The rates of reaction of myoglobin with carbon monoxide at low pH are reported. The pH versus rate profile of these kinetics resembles that found for heme model compounds, revealing an increase in combination rate at low pH. These facts suggest that CO binding by myoglobin changes from a mechanism of “direct ligand association” at pH 5 to a mechanism, similar to that proposed for heme model compounds, which assumes a tetracoordinated intermediate as a result of the protonation of the proximal imidazole.

Ligand binding properties of a great number of simple monomeric hemoproteins with different ligands have been studied in the past. Apart from the large differences in the equilibrium and kinetic constants for the various ligands, such as O₂ and CO, it is well known that the reactivity of different hemoproteins for the same ligand displays great variability. Thus, if we limit ourselves to CO, it may be recalled that the combination rate constant for the binding of this ligand to the ferrous derivative is as low as 5 × 10⁷ M⁻¹ s⁻¹ for horse radish peroxidase (2) and as high as 3 × 10⁸ M⁻¹ s⁻¹ for Chironomus erythrocruein (3). A structural interpretation of these large differences is not available although on a number of findings, they cannot be attributed, solely or even largely, to steric effects encountered on the distal side. In this paper, new experiments to elucidate the structural basis of intrinsic ligand reactivity in simple hemoproteins are reported. The idea behind these experiments originated from a series of investigations on model compounds which have indicated that tetracoordinated ferrous heme, as can be obtained by protonation of the iron-binding imidazole, has a very high “on” constant for CO (4, 5). The central theme of this study is, therefore, to obtain direct information on the rate of CO combination of myoglobin at very acid pH. This is an attempt to gain evidence for the existence and properties of tetracoordinated ferrous iron which could originate from protonation of the proximal imidazole.

EXPERIMENTAL PROCEDURES

Materials — Sperm whale myoglobin was purchased from Serva. Buffers used were sodium phosphate, 10⁻² M, pH 7, or 0.5 M, pH 2.5 to 6 (before mixing).

Design of the pH Jump The experiment consists of (a) rapidly bringing (dithionite reduced) ferrous myoglobin to very low pH values by mixing a neutralized solution of Mb²⁺ (phosphate 10⁻³ M) with a strong buffer (0.5 M phosphate) at pH between 2.5 and 6; (b) measuring the spectrum of the acid form of myoglobin before denaturation has time to occur; (c) measuring the rate of CO combination to this form of the protein, again before denaturation, by mixing myoglobin with an acid buffer containing CO.

RESULTS AND DISCUSSION

Following the acid denaturation of myoglobin, both as the reduced and as the carboxylated derivatives, at different final pH values, we found that the time course of denaturation is complex as previously reported for Hb (6, 7). That ferrous Mb denaturates more rapidly (kₓ₉₅ = 3 s⁻¹ at pH 3) than COMb, and that the rate of the latter process decreased as the CO concentration is increased.

Fig. 1 reports the spectrum of ferrous Mb at time zero after mixing with an acid buffer (final pH 2.4), and for comparison, the spectra of native ferrous Mb and of fully denaturated ferrous Mb. The spectrum at time zero was built from the spectrum of the fully denaturated form plus the optical density changes measured in the flow experiment (corrected for the dead time of the instrument).

It can be seen that the spectrum at time zero is different from that of native Mb at neutral pH; the absorption maximum being shifted to the blue by approximately 10 nm, the maximum extinction being increased by approximately 50%. If we assume that protonation is very fast, i.e. it is completed in the mixing time, the spectrum at time zero represents the optical properties of a modified “acid” myoglobin.

Based on spectra of four-coordinate mesoheme in benzene (8), we expect the four-coordinate protoheme to have a Soret absorption at the same position as that of carboxymyoglobin. The spectrum of the acid form of deoxymyoglobin as shown in Fig. 1 is consistent with this assignment.

Under identical conditions, we have followed combination with CO of the acid form of ferrous Mb, by rapid mixing experiments, in which a known amount of CO was present in the strongly acid buffer so that the protein finds itself confronted with the ligand immediately after the pH jump. The results are as follows: (a) the time course of the absorbance change and the CO concentration dependence show that the observed process reflects a bimolecular reaction over the entire pH range explored; (b) below pH 5, the rate of combination with CO increases as the pH decreases.

The pH dependence of the apparent combination rate constant (k') has been interpreted on the basis of a simple scheme:

\[ H⁺Mb \xrightleftharpoons[K]{l'} Mb + H⁺ \]
\[ CO + F_2 \quad F_1 + CO \]  \[ \quad H⁺MbCO \quad MbCO⁻ \]

where \( l' \) is the rate constant for CO binding to ferrous myoglobin at neutral pH, \( l' \) is the rate constant for CO binding to acid Mb⁺, and the protonation equilibrium \( K \) is assumed to be fast compared to the other two processes.

Under these conditions, the observed second order combination rate \( k \) is given by:

\[ k' = k \frac{K}{[H⁺]} \]

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Reactivity of Ferrous Myoglobin at Low pH

The reactivity of ferrous myoglobin at low pH is characterized by a slow time course with respect to the CO combination. In aqueous cetyltrimethylammonium bromide suspensions of the 3-D-imidazolyllpropyl amide of mesoheme monomethyl ester from Ref. 4, the rate of CO combination according to Scheme 1 can be written in exponential form as:

$$k = \frac{l_1'K + l_2'(H^+)}{K + (H^+)}$$

where $K = (Mb)(H^+)/(MbH^+)$ is the dissociation constant for the proton, $l_1'$ and $l_2'$ are the second order rate constants for the CO combination according to Scheme 1. Equation 2 can be written in exponential form as:

$$k = \frac{l_1'10^{-pk} + l_2'10^{-pk}}{10^{-pk} + 10^{-pk}}$$

The data were fitted with Equation 3 using for $l_1'$ the value of $6 \times 10^3$ M$^{-1}$ s$^{-1}$. A plot of $(\log k - 1.40)$ versus pH for the model compound is shown for reference as a dashed line.

The results reported above suggest the conclusion that the spectral properties of the ferrous myoglobin are profoundly modified when the protein is exposed to strongly acid conditions. In analogy to what was observed on model compounds (4, 5), this spectral change might be attributed to protonation of the imidazole of the proximal histidine residue. Under these conditions, before denaturation (associated with possible release of the heme) has time to occur, the ferrous iron presumably assumes a tetracoordinated structure similar to that observed in model compounds. The tetracoordinated acid form of Mb$^{2+}$ is characterized by a very high second order combination constant with carbon monoxide.

The estimated value for the pure acid Mb$^{2+}$ of $1.4 \times 10^7$ M$^{-1}$ s$^{-1}$ was obtained from the data in Fig. 2 as discussed above. It may be recalled that this asymptotic value is strictly dependent on the assumption that only 1 proton is involved in the transition from neutral to acid myoglobin as shown in Scheme 1. It is noteworthy that the rate of CO combination to the acid Mb$^{2+}$ is comparable with that found for some hemoproteins such as Chironomus erythrocrucrin and the $\beta$ chain of Hb Zurich (9). The determinate correctness of the intrinsic pK of the proximal histidine (3.45) is also dependent on the correctness of the assumptions. This being the case, the $\Delta pK$ with respect to free histidine gives an idea of the strength of the coordination of the iron ion to the proximal residue in myoglobin. It is remarkable that the intrinsic pK for myoglobin (3.45) is almost identical with that of the model compound (3.6) (4), even though the absolute rate constants for myoglobin are about 20 times slower than those of the model compound at all pH values. We plan to extend such studies to other hemoproteins to confirm the hypothesis proposed for myoglobin, and to test whether this idea can assume a general significance for the interpretation of highly reactive forms observed in more complex hemoproteins.

REFERENCES

1. Antonini, E., and Brunori, M. (1971) in Hemoglobin and Myoglobin in Their Reactions with Ligands, North Holland Publishing Co., Amsterdam
2. Brunori, M., Antonini, E., Phelps, C., and Amiconi, G. (1969) J. Mol. Biol. 44, 463-467
3. Amiconi, G., Antonini, E., Brunori, M., Formaneck, H., and Huber, R. (1975) Eur. J. Biochem. 51, 55-58
4. Geibel, J., Chang, C. K., and Traylor, T. G. (1975) J. Am. Chem. Soc. 97, 5924-5926
5. Cannon, J., Geibel, J., Whipple, M., and Traylor, T. G. (1976) J. Am. Chem. Soc. 98, 3385-3396
6. Allis, J. W., and Steinhardt, J. (1970) Biochemistry 9, 2286-2293
7. Cassat, G. C., and Steinhardt, J. (1971) Biochemistry 10, 264-269
8. Brault, D., and Rougee, M. (1974) Biochemistry 13, 4598-4602
9. Gianetti, G. M., di Iorio, E., Antonini, E., Brunori, M., and Winterhalter, K. (1977) Eur. J. Biochem. 75, 267-273

Footnote:
1 The protonation of some residue other than the proximal imidazole with a resulting alteration of the Mb heme pocket toward that of Chironomus hemoglobin cannot be ruled out. However, the spectral change with pH would not be expected to result from simple conformational changes.
Reactivity of ferrous myoglobin at low pH.
G M Giacometti, T G Traylor, P Ascenzi, M Brunori and E Antonini
J. Biol. Chem. 1977, 252:7447-7448.

Access the most updated version of this article at http://www.jbc.org/content/252/21/7447

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/252/21/7447.full.html#ref-list-1
Additions and Corrections

Vol. 252 (1977) 7447–7448

Reactivity of ferrous myoglobin at low pH.

Giorgio M. Giacometti, T. G. Traylor, Paolo Ascenzi, Maurizio Brunori, and Eraldo Antonini

Page 7447, Results and Discussion, line 18

"Increased by approximately 50%" should read "decreased by approximately 25%.

The correct line should read:

It can be seen that the spectrum at time zero is different from that of native Mb at neutral pH, the absorption maximum being shifted to the blue by approximately 10 nm, the maximum extinction being decreased by approximately 25%.

Page 7448, Fig. 1

Fig. 1 should be replaced with the correct figure, shown below:

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.