OXYGEN AND CARBON MONOXIDE BINDING TO MYOGLOBIN FROM THE DOLPHIN FISH *CORYPHAENA HIPPURUS*

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1. Introduction

Complete quantitative studies are necessary to attempt any correlation between molecular structure and functional properties in simple, non-cooperative hemoproteins from various sources. One of the purposes of these studies is to clarify the structural factors which control the reactivity of the heme towards various ligands.

In this communication we report a complete characterization of the equilibrium and kinetics of O_2 and CO binding to the myoglobin from the dolphin fish, *Coryphaena hippurus*, recently purified by Bannister and Bannister [1]. This work seemed of interest from a comparative standpoint, since no kinetic information on fish myoglobin is as yet available.

2. Experimental

Preparation and purification of the myoglobin from *Coryphaena hippurus* were carried out as described by Bannister and Bannister [1].

Oxygen equilibrium experiments (on dithionite or

borohydride reduced) myoglobin were carried out by the method of Rossi Fanelli and Antonini [2]. Kinetic experiments were conducted using a flash photolysis apparatus previously described [3].

The concentration of the protein ($Mb^+(H_2O)$) was obtained using the following value of the extinction coefficient: $E_{mM} = 159$ at $\lambda = 409$ nm [1]. Concentration of the gaseous ligands (O_2 and CO) in solution was calculated from known solubility coefficients.

3. Results and discussion

Oxygen equilibrium data obtained on *Coryphaena* Mb are shown in fig.1. The best fit of the equilibrium experiments yields a Hill coefficient (n_H) of 1.3, and $P_{1/2}$ of 0.9 mm Hg at pH 7.0, 0.2 M phosphate + 10^{-3} M EDTA, and 20°C. Deviation of the shape of the oxygen saturation curve from a simple hyperbola ($n_H = 1$) is not very large, but still would represent a puzzle if all the protein exists as a monomer. Since *Coryphaena* Mb is monomeric [1], and considering the intrinsic difficulties of the experiments (high O_2 affinity and easy formation of met-Mb), we regard the deviation from a simple behaviour non-significant.

The kinetics of O_2 combination was measured by flashing a solution of MbCO in the presence of known concentrations of O_2. Under these conditions the following set of reactions takes place:
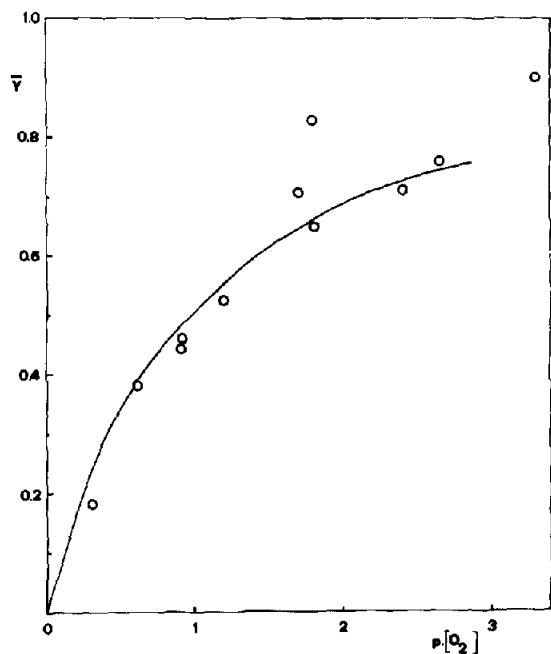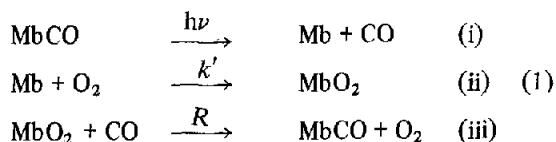
Fig.1. Oxygen dissociation curve of *Coryphaena hippurus* myoglobin obtained at pH 7.0, 0.2 M phosphate + 10^{-3} M EDTA, and 20°C.

$$MbCO \xrightarrow{h\nu} Mb + CO \quad (i)$$

$$Mb + O_2 \xrightarrow{k'} MbO_2 \quad (ii) \quad (1)$$

$$MbO_2 + CO \xrightarrow{R} MbCO + O_2 \quad (iii)$$

Immediately after photolysis, the combination of deoxy Mb with O_2 can be followed (reaction (ii) in scheme 1). The time course of combination conforms to a second order process, and yields a value of the combination velocity constant (k') of 4.6×10^7 M^{-1} sec^{-1} (table 1).

From the kinetics of replacement using CO to displace O_2 (reaction (iii) in scheme (1)), an estimate of the dissociation velocity constant for O_2 may be obtained. The data shown in fig.2 (bottom), obtained at a number of different O_2 and CO concentrations, were treated using the following equation [4]:

$$\frac{1}{R} = \frac{1}{k} + \frac{k'}{l'k} \frac{[O_2]}{[CO]} \quad (2)$$

In conjunction with known values of k' and l' (see below), the O_2 dissociation rate constant (k) was

Table 1
Equilibrium and kinetic constants for the binding of O_2 and CO to myoglobin

	$K(M^{-1})$	$k'(M^{-1}sec^{-1})$	$k(sec^{-1})$	$L(M^{-1})$	$l'(M^{-1}sec^{-1})$	$l(sec^{-1})$
Mb (Horse)	1.3×10^6	1.4×10^7	10.0	2.9×10^7	5.0×10^5	0.017
Mb *(Coryphaena)*	0.6×10^6	4.6×10^7	37^{b} 82^{c}	0.86×10^{7a}	7.2×10^5	0.083
Mb *(Aplysia)*	0.22×10^6	1.5×10^7	70.0	2.5×10^7	5.0×10^5	0.02

[a] Computed from l' and l.
[b] Experimental.
[c] Computed from K and k'.

The following notation is used:

$$Mb + O_2 \rightleftharpoons MbO_2; \qquad K = \frac{(MbO_2)}{(Mb)(O_2)} = \frac{k'}{k}$$

$$Mb + CO \rightleftharpoons MbCO; \qquad L = \frac{(MbCO)}{(Mb)(CO)} = \frac{l'}{l}$$

Fig.3. Dependence of pseudo-first order rate constant on CO concentration. Reaction of Mb with CO at pH 7.0, 0.2 M phosphate + 10^{-3} M EDTA, and 20°C.
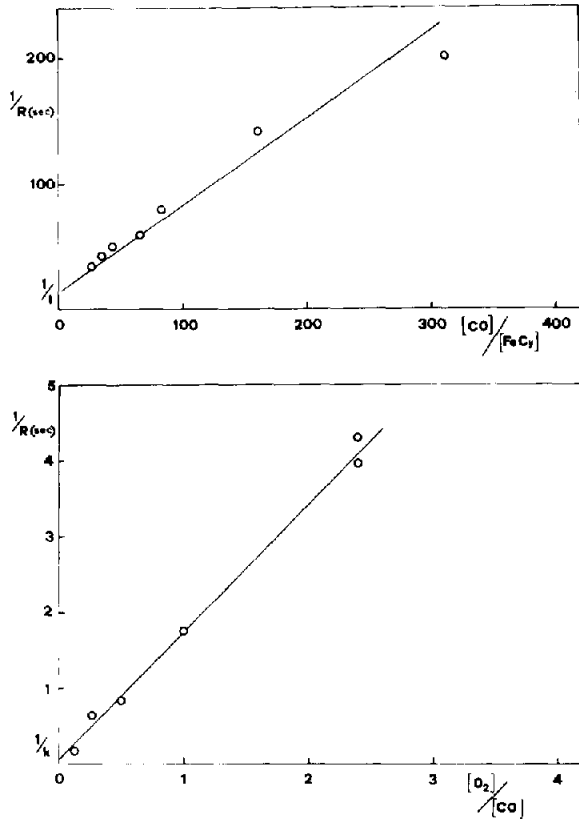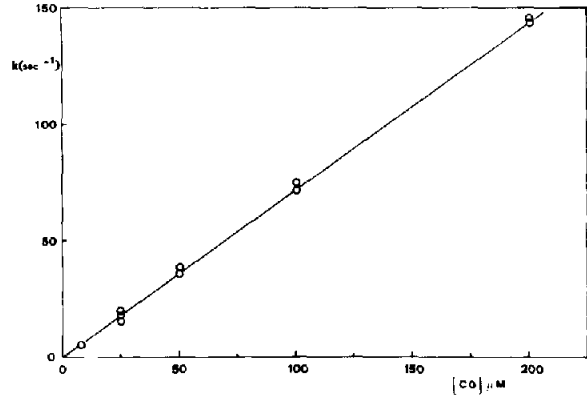
Fig.2. (Top) Displacement on MbCO with ferricyanide obtained at pH 7.0, 0.2 M phosphate + 10^{-3} M EDTA, and 20°C. (Bottom) Displacement on MbO$_2$ with CO obtained at pH 7.0, 0.2 M phosphate + 10^{-3} M EDTA, and 20°C.

obtained both from the intercept and from the slope of the data in fig.2 (bottom).

The kinetics of combination with CO was studied by flash photolysis, at different CO concentrations. The linear dependence of the pseudo-first order rate constant (in sec^{-1}) on the ligand concentration, reported in fig.3, shows that the overall reaction corresponds to a second order process, as expected for simple binding of a ligand. The value of the corresponding second order rate constant is given in table 1.

The rate constant for the dissociation of CO was estimated by replacement with ferricyanide (fig.2, top), using the following equation:

$$\frac{1}{R} = \frac{1}{l} + \frac{l'}{l\,x'} \quad \frac{[CO]}{[FeCy]} \qquad (3)$$

This equation yields the value of l, the CO dissociation rate constant, from the intercept of the plot given in fig.2 (top). The estimated value is given in table 1. In addition the same data yield the second order rate constant for the oxidation of deoxy Mb by ferricyanide, from the slope of the line ($x' = 1.2 \times 10^7$ M^{-1} sec^{-1}).

The results on the equilibrium and kinetic constants for the reaction of *Coryphaena* Mb with O$_2$ and CO are summarized in table 1. These new data are compared with similar results obtained for Mb from horse and Mb from *Aplysia* [5]. Comparison of the data shows clear differences in the values of the constants among Mb from different groups. In particular it is of interest that the Mb from *Coryphaena* is different from the other two myoglobins in the reactivity with both ligands, O$_2$ and CO.

Although it is very difficult to rationalize the observed functional differences in structural terms, it may be of significance that from both O$_2$ and CO the second-order combination velocity constant for *Coryphaena* Mb is higher than that for horse or *Aplysia* Mbs. This finding may be taken to suggest a greater accessibility of the heme crevice to external ligands.

Acknowledgement

References

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