Functional Modulation by Lactate of Myoglobin

A MONOMERIC ALLOSTERIC HEMOPROTEIN*

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The effect of lactate on O2 binding properties of sperm whale and horse heart myoglobins (Mb) has been investigated at moderately acid pH (i.e. pH 6.5, a condition which may be achieved in vivo under a physical effort). Addition of lactate brings about a decrease of O2 affinity (i.e. an increase of P50) in sperm whale and horse heart myoglobins. Accordingly, lactate shows a different affinity for the deoxygenated and oxygenated form, behaving as a heterotropic modulator. The lactate effect on O2 affinity appears to differ for sperm whale and horse heart Mb, logP50 being ~2.00 and ~0.4, respectively. From a kinetic viewpoint, the variation of O2 affinity for both myoglobins can be attributed mainly to a decrease of the kinetic association rate constant for ligand binding.

Myoglobin (Mb)1 is a monomeric hemoprotein present in muscle cells of most vertebrates and invertebrates which reversibly binds O2 with a fairly high affinity (1). Since up to now its function has not been reported to be modulated by environmental conditions, Mb has been thought to display two different roles: (i) to be a reserve supply of O2, and (ii) to facilitate the oxygen flux within a myocyte (2–4).

It has been reported recently that Mb shows ligand-linked tertiary conformational changes (5, 6), suggesting the possibility that the equilibrium between different structural arrangements of the molecule may be affected by non-heme ligands. In the present study, we show that the functional properties of sperm whale and horse Mb s indeed are influenced by lactate, an obligatory product of glycolysis under anaerobic conditions, which appears to play a modulatory role for Mb function, as much as organic phosphates and/or protons (not effective on Mb) influence the function of hemoglobin (7). Lowering of the O2 affinity of Mb by lactate may have relevant physiological consequences, since during a transient tissue hypoxia (such as that which may be induced by a physical effort or diving) the increase of lactate concentration may induce Mb to release O2, thus facilitating its diffusion to mitochondria and helping to keep constant the turnover rate of oxidative phosphorylation.

EXPERIMENTAL PROCEDURES

Sperm whale Mb, horse heart Mb, and MES were obtained from Sigma. Lactate has been purchased as L-(-)-lactic acid from Fluka Chemie AG (Buchs, CH) and used without further purification. All chemicals were of reagent grade and used without further purification. 2-cm path length observation cell interfaced to a desk-top computer for pulse experiment (9).

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higher affinity of lactate for deoxyMb than for MbO₂ (is rendered more stringent by the observation that Therefore, the decrease in O₂ affinity (is the existence of a ligand-linked structural change such that lactate binds oxygenated and deoxygenated Mb with different affinity, according to the following minimum reaction scheme (Scheme I) (13)
\[
\begin{align*}
K_{d}^{o} & \quad \text{Mb} + O_{2} \rightleftharpoons \text{MbO}_2 \\
+ & \quad \rightarrow \\
L & \quad L \\
K_{d}^{L} & \quad \| \\
\text{MbL} + O_{2} & \rightleftharpoons \text{MbO}_2 L
\end{align*}
\]
where \(K_{d}^{o}\) and \(K_{d}^{L}\) are the equilibrium dissociation constants for oxygen binding to Mb in the absence and in the presence of saturating amounts of lactate, respectively, and \(K_{d}^{o}\) and \(K_{d}^{L}\) are the equilibrium dissociation constants of lactate for deoxy- and oxyMb, respectively. The minimum reaction Scheme I implies a 1:1 Mb: lactate stoichiometry, which is not unequivocally proved at this stage, even though it is likely. Under this assumption, the effect of lactate concentration on the observed oxygen equilibrium dissociation constant for Mb (is \(K_{d}^{o}(p)\) is then (13)
\[
K_{d}^{o}(p) = K_{d}^{o}((1 + [L]K_{d}^{o}(1 + [L]K_{d}^{L}))
\]
(Eq. 1)
Therefore, the decrease in O₂ affinity (is \(P_{50}\) or of \(K_{d}^{o}(p)\)) observed upon addition of lactate (see Fig. 1) is due to a higher affinity of lactate for deoxyMb than for MbO₂ (is \(K_{d}^{o} > K_{d}^{L}\), from which it stems that \(K_{d}^{o} > K_{d}^{L}\) (see Scheme I).

The analysis of data reported in Fig. 1 according to Equation 1 is rendered more stringent by the observation that \(K_{d}^{L}\) can be obtained independently, since addition of lactate brings about a spectroscopic change of the absorption spectrum of sperm whale and horse heart MbO₂, which is characterized by a decrease of the extinction coefficient at 418 nm (see Fig. 2A). Values of \(K_{d}^{o}\) turn out to be essentially the same (within the experimental error) for sperm whale and horse heart Mb, and the same can be said for \(K_{d}^{L}\), that is the affinity for oxygen of the two Mb in the absence of lactate. Therefore, application of Equation 1 to data reported in Fig. 1, keeping fixed the values of parameters determined independently, allows a complete thermodynamic description of the effect of lactate on the O₂ binding properties of both Mbs (see Table I). It comes out that the different functional effect of lactate on sperm whale and horse heart Mb (see Fig. 1) is totally related to a different affinity of lactate for the unliganded form of the two myoglobins.

As shown in Table I, the dissociation rate constant of O₂ from both Mbs is unaffected by the presence of lactate (up to 0.2 M concentration), and, from kinetics of O₂ binding to horse heart Mb, it turns out that the smaller value of \(K_{d}^{L}\) with respect to \(K_{d}^{o}\) may be wholly attributable to a decrease of the association rate constant for O₂ (Fig. 2B). Such behavior may indicate that either (i) lactate represents itself as a physical impairment for the dynamic approach of O₂ to the heme and/or (ii) a lactate-induced structural change of the protein may result in an increase of the activation free energy for the ligand binding pathway.

These considerations open the question of the location of the possible site of lactate binding to Mb. The inspection of the
crystal structure of different derivatives of sperm whale Mb has shown an anion (e.g. sulfate) binding site at hydrogen bonding distances from HisE7, ArgCD3, and ThrE10 residues (14). Soaking of sperm whale oxygenated Mb crystals in 0.1 M lactate at pH 6.5 did not result in significant changes of the heme absorption features (see Fig. 2). However, lack of lactate binding to the crystalline protein may be in keeping with the moderate affinity of lactate for MbO2 (see Table I) as well as with the high ionic strength of the mother liquor. In fact, crystals are grown in 1 M MES, at pH 6.5. We thank Prof. M. Bolognesi, Prof. M. Castagnola, and Prof. M. Marchetti for fruitful discussions and Prof. M. Brunori for the kind hospitality to carry out stopped-flow experiments at the Dept. of Biochemical Sciences “Alessandro Rossi Fanelli” of the University of Rome “La Sapienza.”

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Table I

| Thermodynamic and kinetic parameters for O2 and lactate binding to sperm whale and horse heart Mbs in 0.1 M MES, at pH 6.5 and 20 °C (see Scheme I) |
|---------------------------------------------------------------|
| Thermodynamic parameters                                      |
| KαO2 (m)                                                      | 4.8 × 10^{-7}       | 5.0 × 10^{-7} |
| KβO2 (m)                                                      | 5.0 × 10^{-7}       | 1.4 × 10^{-6} |
| KαL (m)                                                      | 2.6 × 10^{-2}       | 2.6 × 10^{-2} |
| KβL (m)                                                      | 2.5 × 10^{-3}       | 9.1 × 10^{-3} |
| Kinetic parameters                                            |
| Without lactate                                              |
| k (s^{-1})                                                    | 2.9 × 10^{3}        | 2.5 × 10^{7}  |
| k (s^{-1})                                                    | 1.8 × 10^{1}        | 1.2 × 10^{1}  |
| With 0.2 M lactate                                            |
| k (s^{-1})                                                    | 3.7 × 10^{6} a      | 8.6 × 10^{6}  |
| k (s^{-1})                                                    | 1.8 × 10^{3}        | 1.2 × 10^{1}  |

a The value of k' for O2 binding to sperm whale Mb, in the presence of 0.2 M lactate, was calculated on the basis of the following relationship: k' = k/ KβO2.