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Interpretation of the Binding of Carbon Monoxide to Hemoglobin Under Photodissociating Conditions

(theoretical/ligand/protein-modulated interactions/light)

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ABSTRACT **An interpretation is given of recent experiments by Brunori *et al.* on the binding of carbon monoxide by hemoglobin under photodissociating conditions. It is shown that their results follow directly from scalable models for hemoglobin in which the protein-modulated interactions are separable from the ligand binding.**

Separation of the various elements involved in the cooperative binding of ligands is an objective of many experimental investigations of hemoglobin. One approach to the problem is to introduce molecular alterations that are presumed to have relatively localized effects. An ideal perturbation of this type is provided by light whose primary effect in the long-wavelength region is on the iron–ligand bond. Brunori *et al.* (1) have recently studied the binding of CO by myoglobin (Mb) and hemoglobin (Hb) under photodissociating conditions. They reported that although the Mb data can be understood easily, the interpretation of the behavior of Hb raises significant difficulties. We wish to suggest that the Hb results in the presence of light are a direct consequence of scalable models for hemoglobin in which the protein-modulated interactions are separable from the ligand binding. In what follows, we summarize the experimental results for Hb and Mb, review the interpretation of the Mb data, and demonstrate by use of a model of the Monod–Wyman–Changeux type how to account for the Hb observations.

For both Mb and Hb, the partial pressure of CO required to half-saturate the macromolecule ($p_{1/2}$) was found by Brunori *et al.* (1) to increase linearly with light intensity, and the shape of the binding curve (fractional saturation as a function of the logarithm of the CO partial pressure) was unaffected by light. The apparent enthalpy of binding (as obtained from the temperature dependence of log $p_{1/2}$) varied in Mb and Hb from $\Delta H \simeq -11.5$ kcal/mol in the dark to a maximum of +7 kcal/mol with increasing light intensity; the limiting value is equal to the activation energy of the "on" process for binding of CO by Mb. For Hb, the Bohr effect (as measured by $\partial \log p_{1/2}/\partial$pH) was not altered by light.

A simple interpretation can be given of the Mb results, as pointed out by Brunori *et al.* (1). In the presence of light, the "on" constant, k^I_{on}, is the same as in the dark ($k^I_{on} = k_{on}$), while the effective "off" constant, k^I_{off}, is expected to increase linearly with intensity I ($k^I_{off} = k_{off} + aI$, where a is the appropriate factor); the unsuperscripted constants refer to quantities in the absence of light and those with superscript I to the corresponding quantities in the presence of light. The association equilibrium constant at light intensity I, K^I, can then be written

$$K^I = \frac{k_{on}}{k_{off} + aI} = K(1 + aI/k_{off})^{-1} \quad [1]$$

Since $p^I_{1/2}$ for Mb is $[K^I]^{-1}$, we have

$$p^I_{1/2} = [K^I]^{-1} = p_{1/2}\frac{K}{K^I} = p_{1/2}\left(1 + \frac{aI}{k_{off}}\right) \quad [2]$$

It can be seen from Eq. 2 that $p^I_{1/2}$ increases linearly with light intensity, in agreement with experiment. Moreover, the behavior of ΔH is explained; since for sufficiently large I, $aI \gg k_{off}$, the measured enthalpy of binding should approach the activation enthalpy for the "on" reaction.

To interpret the Hb results, we use the Monod–Wyman–Changeux model in its simplest form (2). It is conveniently expressed in terms of the generating function (3), $\Xi(p)$,

$$\Xi(p) = L(1 + cKp)^4 + (1 + Kp)^4 \quad [3]$$

with the fractional saturation $\langle y_{CO}\rangle$ at CO partial pressure p given by

$$\langle y_{CO}\rangle = \frac{1}{4}p\frac{\partial}{\partial p}[\ln\Xi(p)] \quad [4]$$

In Eq. 3, L is the allosteric constant, c is the binding constant ratio for the two quaternary structures, and K is the ligand binding constant for the oxy quaternary structure. The essential point in applying Eq. 3 to the present problem is the assumption that the parameters L and c are determined by the protein, while K is an intrinsic parameter of the heme group. A detailed model for hemoglobin that satisfies this assumption has been proposed recently (3). Although the generating function for the model is more complicated than Eq. 3, an analysis corresponding to that given here can be made. Factors in the model analogous to c depend on the strengths of certain salt bridges, while the quantity corresponding to L is a function of salt-bridge strengths and other nonbonded interactions.

If light alters only K in Eq. 3, in a manner analogous to myoglobin (i.e., if it is valid to replace K in Eq. 3 for Hb in

Abbreviations: Hb, hemoglobin; Mb, myoglobin.

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light by K^I, as given by Eq. 1) we find by use of Eqs. 1, 3, and 4 that $p^I{}_{1/2}$ for Hb is given by

$$p^I{}_{1/2} = p_{1/2}(1 + aI/k_{off}) \quad [5]$$

Eq. 5, which has the same form as Eq. 2, though $p_{1/2}$ and a have different values, accounts for the linear variation of the affinity with light intensity. Moreover, the shape of the saturation curve ($\langle y_{CO} \rangle$ versus log p), including the value of the Hill constant n, is unaltered by light, because a change of K in Eq. 3 at constant L and c leads only to a shift ("scaling") of the binding curve (2). Further, it is easily shown that this model satisfies a requirement for the invariance of the binding curve of the type discussed by Brunori *et al.*; namely, the additive factor by which each of the four Adair dissociation constants for Hb changes in the presence of light is proportional to the value of the constant itself. The observed invariance of the Bohr effect in Hb with illumination follows if a/k_{off} is independent of pH. The fact that k_{off} is independent of pH is physically reasonable and can be justified in terms of a detailed model (3). Also, there is experimental evidence (4) for the case of O_2 binding by Hb that the last off-constant (k_4), which corresponds most closely to k_{off}, is not a function of pH. To examine the consistency of this interpretation, it would be useful to determine the change with pH of the slope of the plot of $p^I{}_{1/2}$ versus light intensity: with a/k_{off} independent of pH, Eq. 5 predicts that the slope decreases with increasing pH and that the ratio of the slopes at two different pH values is equal to the ratio of the corresponding $p_{1/2}$ values of Hb in the dark.

The dependence of ΔH on light intensity for Hb is more difficult to analyze quantitatively, but it is not unreasonable to expect the same qualitative behavior as Mb. In the Monod–Wyman–Changeux model, $p_{1/2}$ can be written as

$$p^I{}_{1/2} = \frac{f(L,c)}{K^I} \quad [6]$$

where $f(L,c)$ is the appropriate function of the constants obtained from Eq. 3. Thus, if $f(L,c)$ is independent of temperature, the apparent enthalpy at high light intensities should approach the activation enthalpy of the "on" reaction exactly as in Mb. Although unequivocal data are not available, $f(L,c)$ is expected to have only a weak temperature dependence from Roughton's measurements which show that the heat of reaction for the binding of oxygen is nearly independent of saturation (5,6).

The above development shows that a model of the Monod–Wyman–Changeux type provides a consistent interpretation of the recent experimental data of Brunori *et al.* (1). The essential element in the analysis is complete separation of the interaction of the protein subunits from the off reaction, which is affected by light. Consequently, other models that preserve this element and lead to scaling [e.g., a model of the Pauling type with the subunit interaction constant independent of light (7)] would yield corresponding results. Also, models (3, 8) that take account of the inequivalence of the α and β chains of Hb are consistent with the measurements of Brunori *et al.* (1), although deviations from linearity of the $p^I{}_{1/2}$ versus I curve can result; for the parameter values used in the models (3, 8), the deviations occur at very low light intensity and appear to be below the limits of experimental error. However, because of the significance of the Hb results, including their pH and temperature dependence, the more detailed measurements promised by Brunori *et al.* will be of great interest in examining further the separability and characteristics of the various contributions to ligand binding.

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