Oxygenation of partially oxidized human hemoglobin

Dear Sir,

In a recent paper, Marden et al. (1991) reported oxygen equilibrium curves of hemoglobin at various oxidation levels, with CN^- bound to the ferric hemes. The authors rationalized their experimental results in terms of a modified Monod-Wyman-Changeux (MWC) model in which the allosteric equilibrium of hemoglobin tetramers with k ferric subunits and i dioxygen molecules bound results to be:

$$T_i^k/R_i^k = Lc^i m^k, \qquad (1)$$

where L is the usual allosteric parameter of MWC model, $c = K_R/K_T$, and m expresses the shift in the allosteric equilibrium caused by the presence of a ferric heme; the model, moreover, assumes the distribution of tetramers having zero to four ferric hemes to be statistical and resulting from the well known binomial distribution. The oxygen saturation function of each partially ferric species is calculated using a two state formalism in which the allosteric coefficient is given by Eq. 1. The overall oxygen saturation function is finally obtained by summing the oxygen saturation functions relative to each partially ferric species weighted by the fraction of each species and by the number of ferrous hemes per species.

The experimental data were obtained at 60 μM protein concentration in heme (except at 90% oxidation, where 300 μM protein concentration was used); the fitting in terms of the model gives $L = 1.3 \times 10^6$; $K_R = 0.33$ mmHg, and $m = c = 0.0066$. Having obtained $m = c$, the authors conclude that the ferric ligand CN^- has, on the allosteric equilibrium of hemoglobin, an effect identical to that of oxygen; moreover, the presence of ferric hemes bound with CN^- does not alter the oxygen dissociation constants relative to the T or to the R state (K_T and K_R, respectively).

Although the experiments have been performed at a rather low protein concentration, the presence of a dimer $\rightleftarrows$ tetramer equilibrium has not explicitely taken into consideration in the model. The presence of dimers has two main consequences.

(a) A minor one related to the higher oxygen affinity of dimers that affects the oxygen equilibrium curves (Ackers et al., 1975); it must be pointed out that assuming a tetramer $\rightleftarrows$ dimer association constant of $4 \times 10^5\ M^{-1}$ (Chu and Ackers, 1981), ~18.5% of the subunits is found to be in dimeric form when the total protein concentration is 60 μM in heme; indeed, Marden et al. (1991) need a large contribution (10%) of non-allosteric forms such as dimers to fit their kinetic results.

(b) The second, major, consequence concerns the distribution of ferric hemes within the tetramers; in fact, the dimer $\rightleftarrows$ tetramer equilibrium constant depends markedly upon the presence of ligands (in the unligated T state the dimer $\rightleftarrows$ tetramer association constant is increased by at least four orders of magnitude with respect to the fully ligated state; Chu and Ackers, 1981); this, in turn, makes the tetramers lifetime depend upon their ligation state, bringing about a nonstatistical distribution of ferric hemes within the hemoglobin tetramers that also depends upon the oxygenation state.

Our group has recently published a paper on the effect of partial auto-oxidation on the oxygen equilibrium curves of hemoglobin using H_2O as a ferric ligand (Cordone et al., 1990). The data were analyzed with a modified MWC model also based on Eq. 1. In our model, however, the dimer $\rightleftarrows$ tetramer equilibrium was explicitely taken into account and a ligation dependent c value was used. As a consequence of the species readjustment due to the fast dimer $\rightleftarrows$ tetramer equilibrium a distribution of ferric hemes within the hemoglobin tetramers markedly differing from the statistical one and depending upon oxygenation was found.

To further investigate this point we have taken the experimental points from Fig. 1 of the paper of Marden et al. (1991) (relative to oxygen equilibrium curves performed at various oxidation levels, with cyanide bound to the ferric hemes) and analyzed them within the framework of our model. Fittings are reported in Fig. 1; their quality is excellent, and rms deviations are 7×10^{-2} on log $Y/1\text{-}Y$ and 8×10^{-3} on Y. Parameters values are reported in the second column of Table 1; although these values cannot be considered definitive (since the entire set of experimental data is not available to us) we think that some interesting conclusions can be drawn.

(a) A high L value and a low K_R value is obtained; this is due to the well known compensation between these two parameters.

(b) A value of $m/c_0 \approx 1.5$ is obtained; this indicates that, in agreement with Marden et al. (1991), each CN^- ion bound makes approximately the same contribution as an oxygen in shifting the allosteric equilibrium towards the R state.

(c) ligation dependent c values are needed to fit the experimental data. The c_i values reported in Table 1 refer to the tetramers, since dimers are assumed to be in R conformation and to bind oxygen noncooperatively with an oxygen dissociation constant equal to K_R (Cordone et al., 1990). Very interestingly, a c_3 value of 1 is obtained; this implies that the presence of three bound CN^- ions, probably due to the linear axial geometry of the heme-CN^- complex, introduces stresses in the tertiary structure of T state hemoglobin (Moffat et al., 1979) such to make $K_T = K_R$.

The above points indicate that one must be very careful in extrapolating properties of oxygenated hemoglobin from data obtained using cyanomet-hemoglobin as a model, especially for what concerns triply ligated species.

The distribution of ferric subunits within fully deoxygenated and fully oxygenated hemoglobin tetramers is reported in Fig. 2 as a function of the total fraction of oxidized protein and compared with the binomial distribution assumed by Marden et al. (1991). Data in Fig. 2 show that such distributions, indeed, differ from the statistical one and depend upon oxygenation.

We have applied the above approach to analyze also the oxygen equilibrium curves performed at various oxidation levels with fluoride bound to the ferric hemes (data reported in Fig. 2 of the paper by Marden et al. [1991]). The quality of fitting is again excellent, and the rms deviation is 5×10^{-2} on

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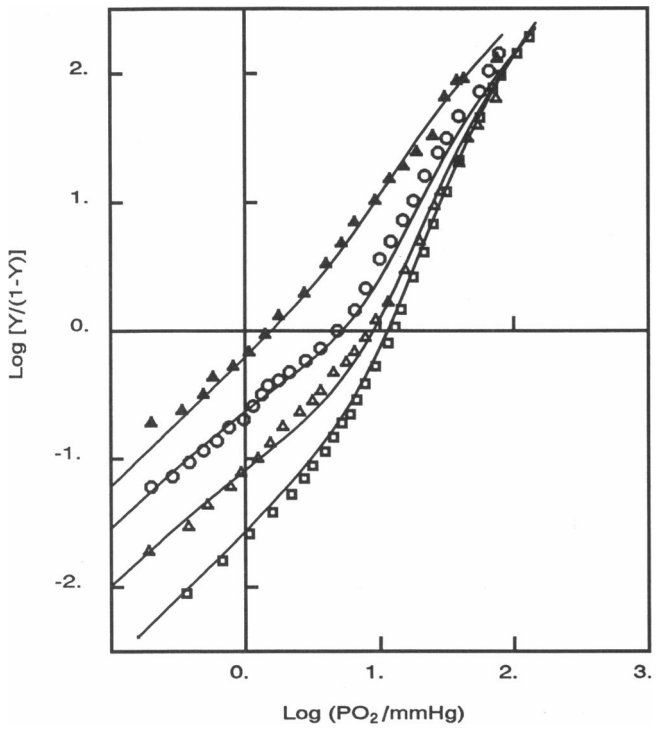

FIGURE 1 Hill plots relative to oxygen equilibrium curves (data have been taken from Fig. 1 of the paper of Marden et al., 1991) obtained in the presence of various amounts of oxidized hemoglobin. (□) $f(Hb^{3+} - CN^-) = 0.03$; (△) $f(Hb^{3+} - CN^-) = 0.30$; (○) $f(Hb^{3+} - CN^-) = 0.50$; (▲) $f(Hb^{3+} - CN^-) = 0.80$. The continuous lines are the best fits obtained using the model described in the paper of Cordone et al. (1990) and the set of parameters reported in Table 2.

$\log [Y/(1-Y)]$; parameters values are reported in the third column of Table 1. They differ from those obtained in the presence of CN^- only for m/c_0 (3.5 for F^- and 1.5 for CN^-) and for c_3 (0.015 for F^- and 1 for CN^-); these differences can be put in relation with the smaller dimensions of fluoride ion with respect to cyanide ion.

We like to end by stressing that, due (*a*) to the peculiar role that dimerization plays in "stirring" tetrameric species and (*b*) to the longer lifetime of unligated T state tetramers, a particular care must be taken in the analysis of experimental results using models not taking explicitely into account this effect.

TABLE 1 Values of the parameters obtained from the fitting to the entire set of data points relative to oxygenation measurements in the presence of various amounts of cyanomet- and fluoromet-hemoglobin

Parameter	CN^-	F^-
L ($\times 10^7$)	2 ± 1	2 ± 1
c_0	0.004 ± .0002	0.004 ± .0002
c_1	0.003 ± .001	0.004 ± .0005
c_2	0.012 ± .002	0.010 ± .005
c_3	1 ± .5	0.015 ± .004
m	0.006 ± .0002	0.011 ± .0004
K_R (mmHg)	0.173 ± .002	0.170 ± .002

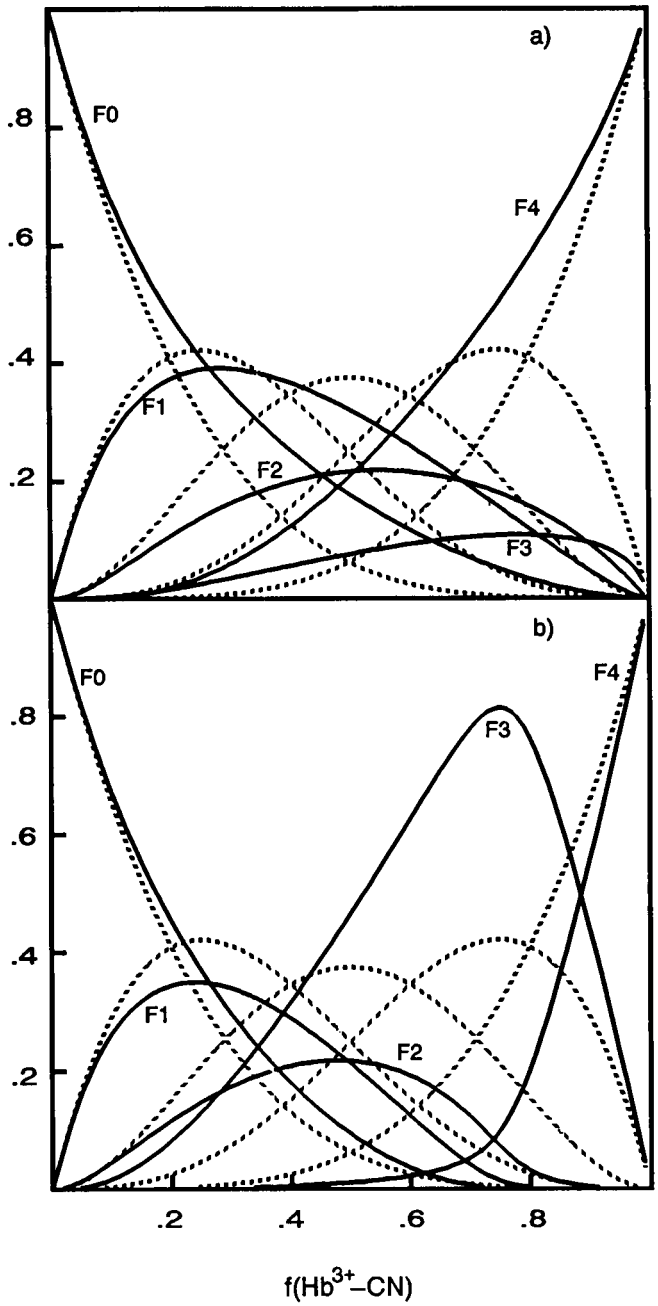

FIGURE 2 Distribution of ferric subunits within fully deoxygenated (*a*) and fully oxygenated tetramers (*b*) as a function of the total fraction of $Hb^{3+} - CN^-$. The quantities F_k indicate the fraction of tetramers with k ferric subunits. The continuous lines are the distributions obtained using the model described in the text and the broken lines are the statistical binomial distributions of the several species. Hemoglobin concentration is 6×10^{-5} M in heme.

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