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A THERMODYNAMICAL MEASURE OF COOPERATIVITY:

APPLICATION TO HEMOGLOBIN

by

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ABSTRACT

A comparative analysis of the heat requirements for di-oxygen exchange is made for hemoglobin and myoglobin, the latter taken as the prototype of the vertebrate hemoglobin's ancestor. It is shown that cooperativity manifests itself also in terms of energy utilization.

Key-words: Cooperativity; Macromolecules.

1 INTRODUCTION

Usually cooperativity is measured by Hill's parameter \underline{n} , introduced in 1910 to describe the oxygen-hemoglobin equilibria¹. Since $n = \frac{\partial \ln \frac{Y}{1-Y}}{\partial \ln p_{O_2}}$, it suffers the limitation of being a local parameter. Recently², we have proposed the mean allosteric free energy, $G_{34,12}^0$, as a more general measure of cooperativity. It was possible to find an algebraic relation between n_m (\underline{n} at the mean oxygen pressure) and four parameters: p_m (mean partial pressure), k_1 (the first Adair constant) $G_{41,32}^0$ (the asymmetry free energy^{3,4}) and $G_{34,12}^0$. It was also possible to show that the leading term in the analytical expression is $G_{34,12}^0$.

Hemoglobin is presumed to have had a non-cooperative myoglobin-like 5×10^8 years old ancestor^{5,6} and the same should be true for other cooperative molecules developed through evolution. From an evolutionary point of view, therefore, it would be important to be able to measure cooperativity in relation to ancestral molecules.

One way of doing this is to compare the energy spent, for example, in the exchange of a given amount of oxygen by hemoglobin and by myoglobin, chosen as the prototype of a monomeric ancestor. Either this or the relative measure of the amount of oxygen exchanged for a given expenditure of energy would be important indexes of cooperativity as an evolutionary artifact.

This paper is a program of how such a measure can be profitably done. Significantly, it will also show the importance of the non-equivalence of sites. This functional asymmetry, known for a number of years^{7,8,9} and now confirmed¹⁰ by the

evidence that $\text{Hb}(\text{O}_2)_2$ is mainly $\alpha\text{oxy}\beta\text{deoxy}$, has also been invoked² to explain the remarkable thermal stability of human A hemoglobin.

2 THEORY

The inclusion of the functional non-equivalence of the α and β chains in the description of the Hb/O_2 equilibria is made difficult by an enormous increase in the number of unknown variables¹¹. However, a simple model that retains the basic features of the problem and lacks most of its complications may be used to simplify the issues.

Let us consider the mock dimeric protein $\delta\gamma$, each δ and γ subunit being similar to the myoglobin molecule and to the α and β chains as well. This dimer will be compared to a mock myoglobin-like monomer.

Suppose one liter of a monomer sample undergoes a variation of fractional saturation, ΔY^M ; the corresponding heat exchange ΔH^M is given by

$$\Delta H^M = [\text{S}]_T \Delta Y^M \Delta H^0 \quad (1)$$

where ΔH^0 is the standard enthalpy of oxygen binding of the monomer and $[\text{S}]_T$ is the total concentration of active sites.

If the dimer sites interact cooperatively, the relation between the heat exchange of one liter of the dimer sample, ΔH^D , and the fractional saturation variation ΔY^D is somewhat more

complicated:

$$\Delta H^D = [\bar{S}]_T \Delta Y^D \Delta H_1^0 + \Delta [\bar{D}(O_2)_2] H_{21}^0 \quad (2)$$

The total concentration of active sites is considered to be the same. ΔH_1^0 and ΔH_2^0 are the stepwise standard enthalpies of binding of the first and the second oxygen molecules to the dimer; $H_{21}^0 = \Delta H_2^0 - \Delta H_1^0$ is the enthalpy of allosteric interaction; $[\bar{D}(O_2)_2]$ is the concentration of the species $\delta\gamma O_2$ and $\Delta [\bar{D}(O_2)_2]$ its variation during oxygenation. $[\bar{D}(O_2)_2]$ may be given analitically as a function of Y^D , but for our present purposes it suffices to know that $\Delta [\bar{D}(O_2)_2] \gtrsim 0$ if $\Delta Y^D \gtrsim 0$.

To compare ΔH^D with ΔH^M we subtract equation (1) from equation (2):

$$\Delta H^D - \Delta H^M = [\bar{S}]_T (\Delta Y^D \Delta H_1^0 - \Delta Y^M \Delta H^0) + \Delta [\bar{D}(O_2)_2] H_{21}^0 \quad (3)$$

We need now a relation between ΔH_1^0 and ΔH^0 . We will make the reasonable assumption that the oxygenation enthalpy of the monomer, ΔH^0 , is equal to the oxygenation enthalpy of the species $\delta\gamma O_2$ ($\Delta H_{\gamma\delta}^0$), for the purpose of the dimer model is to reproduce some of the known facts about hemoglobin's cooperativity¹², and, in this sense, $\Delta H_{\gamma\delta}^0$ is the enthalpy of oxygenation of dimer molecules free of constraints in the $\delta - \gamma$ interface (hence, similar to monomer molecules).

Consider the equations

$$\Delta H_1^0 = n_\delta \Delta H_\delta^0 + n_\gamma \Delta H_\gamma^0 \quad (4)$$

$$H_{21}^{\circ} = H_{\delta\gamma}^{\circ} + (n_{\delta} - n_{\gamma}) (\Delta H_{\gamma}^{\circ} - \Delta H_{\delta}^{\circ}) \quad (5)$$

$$\Delta H_{\gamma\delta}^{\circ} = \Delta H_{\delta\gamma}^{\circ} + \Delta H_{\delta}^{\circ} - \Delta H_{\gamma}^{\circ} \quad (6)$$

$$n_{\delta} + n_{\gamma} = 1 \quad (7)$$

where n_{δ} and n_{γ} are the occupation numbers of the δ and γ subunits in the mono-oxygenated species, $H_{\delta\gamma}^{\circ} = \Delta H_{\delta\gamma}^{\circ} - \Delta H_{\gamma}^{\circ}$ is the site-specific enthalpy of allosteric interaction, $\Delta H_{\delta}^{\circ}$ and $\Delta H_{\gamma}^{\circ}$ are the standard enthalpy changes of the reactions $\delta\gamma + O_2 \rightarrow \delta O_2\gamma$ and $\delta\gamma + O_2 \rightarrow \delta\gamma O_2$. Equations (4) and (5) were already discussed²; equation (6) is the statement of enthalpy conservation between the initial (D) and final (D(O₂)₂) states of dimer oxygenation.

From $\Delta H^{\circ} = \Delta H_{\gamma\delta}^{\circ}$ and equations (4) through (7) we finally obtain the desired relation between ΔH° and ΔH_1° :

$$\Delta H_1^{\circ} - \Delta H^{\circ} = -H_{\delta\gamma}^{\circ} + n_{\gamma} (\Delta H_{\gamma}^{\circ} - \Delta H_{\delta}^{\circ}) \quad (8)$$

Also, the following inequalities have been already discussed²:

$$-H_{\delta\gamma}^{\circ} > 0 \quad (9)$$

$$\Delta H_{\gamma}^{\circ} - \Delta H_{\delta}^{\circ} > 0 \quad (10)$$

Hence, the two terms on the right-hand side of equation (8) are both positive.

We will now make a comparison of the thermodynamic efficiencies of the dimeric (D) and monomeric (M) species under three conditions:

i) no restriction with respect to heat and oxygen exchange variations is made upon the dimer and monomer samples:

ii) the enthalpy variations of both samples are considered to be the same ($\Delta H^D = \Delta H^M$);

iii) the variations of fractional saturation (and, therefore, the quantities of oxygen liberated by both samples) are considered to be the same ($\Delta Y^D = \Delta Y^M = \Delta Y$).

Considering first the unrestricted case, we substitute equation (8) in (3) and after some manipulation we have

$$\chi = \frac{\Delta H^D}{[\bar{S}]_T \Delta Y^D} - \frac{\Delta H^M}{[\bar{S}]_T \Delta Y^M} = -H_{\delta Y}^0 + n_Y (\Delta H_Y^0 - \Delta H_{\delta}^0) + \frac{\Delta [\bar{D}(O_2)_2]}{[\bar{S}]_T \Delta Y^D} H_{21}^0 \quad (11)$$

The function defined by equation (11), which will be called the comparative efficiency (χ), is a measure of the excess of heat required by the monomer sample (as compared to the dimer one) per mole of released or uptaken oxygen. We have shown² that in order to mimicry the thermal behaviour¹³ of hemoglobin A, the first term in the RHS of equation (5) is overwhelmed by the second term, making $H_{21}^0 > 0$. Recalling inequalities (9) and (10) it is clear that χ is always a positive quantity.

Let us consider now condition ii), i.e., $\Delta H^0 = \Delta H^M$. By introducing again in equation (3) relation (8), and after some algebra we obtain a function which will be referred to as the relative efficiency (ρ)

$$\rho = \frac{\Delta Y^D - \Delta Y^M}{\Delta Y^D} = \frac{H_{\delta Y}^0 - n_Y (\Delta H_Y^0 - \Delta H_{\delta}^0)}{\Delta H^0} - \frac{\Delta [\bar{D}(O_2)_2]}{[\bar{S}]_T \Delta Y^D} \frac{H_{21}^0}{\Delta H^0} \quad (12)$$

By the same reasoning used before we are lead to the conclusion that ρ is also positive. That means that if the dimer and monomer samples undergo equal enthalpy variations, then the former sample exchanges more oxygen than the latter. In fact, the comparative and relative efficiencies are related by

$$\chi = - \Delta H^{\circ} \rho \quad (13)$$

Finally let us consider condition iii). Substituting in equation (3) relation (8), and $\Delta Y^D = \Delta Y^M = \Delta Y$, we obtain

$$\Delta H^D - \Delta H^M = [\bar{S}]_T \Delta Y (-H_{\delta}^{\circ} \gamma + n_{\gamma} (\Delta H_{\gamma}^{\circ} - \Delta H_{\delta}^{\circ})) + \Delta [\bar{D}(O_2)_2] H_{21}^{\circ} \quad (14)$$

This function is positive if oxygen is being uptaken and negative if oxygen is being released. This means that if both samples exchange equal amounts of oxygen the dimer exchanges less heat than the monomer. The indexes χ and ρ are related to $\Delta H^D - \Delta H^M$ be the equations

$$\chi = \frac{\Delta H^D - \Delta H^M}{[\bar{S}]_T \Delta Y} \quad (15)$$

and

$$\rho = - \frac{\Delta H^D - \Delta H^M}{[\bar{S}]_T \Delta Y \Delta H^{\circ}} \quad (16)$$

3 THE HEMOGLOBIN CASE.

We turn now to our specific system, that of tetrameric hemoglobins. We will compare the Hb/O₂ equilibrium with the Mb/O₂ one, again under the three theoretical conditions (i), (ii) and (iii). For condition (iii) ($\Delta Y^{\text{Hb}} = \Delta Y^{\text{Mb}} = \Delta Y$) we obtain the equation

$$\begin{aligned} \Delta H^{\text{Hb}} - \Delta H^{\text{Mb}} = & [\text{S}]_{\text{T}} \Delta Y \left(\frac{\Delta H_1^{\circ} + \Delta H_2^{\circ}}{2} - \Delta H^{\circ} \right) + \\ & + \Delta \left([\text{HbO}_2] + [\text{Hb}(\text{O}_2)_3] \right) \frac{\Delta H_1^{\circ} - \Delta H_2^{\circ}}{2} + \\ & + \Delta \left([\text{Hb}(\text{O}_2)_4] + \frac{[\text{Hb}(\text{O}_2)_3]}{2} \right) H_{34,12}^{\circ} + \\ & - \frac{\Delta [\text{Hb}(\text{O}_2)_3]}{2} H_{41,23}^{\circ} \end{aligned} \quad (17)$$

analogous to (14). In this equation the ΔH_i° ($i = 1, 2, 3, 4$) are the stepwise standard enthalpies of binding the i^{th} oxygen molecule to hemoglobin, ΔH° is the myoglobin standard oxygenation enthalpy, $H_{34,12}^{\circ} = (\Delta H_3^{\circ} + \Delta H_4^{\circ}) - (\Delta H_1^{\circ} + \Delta H_2^{\circ})$, $H_{41,23}^{\circ} = (\Delta H_4^{\circ} + \Delta H_1^{\circ}) - (\Delta H_2^{\circ} + \Delta H_3^{\circ})$, and the other terms have the same meaning as in equation (14). The independent variables are the four ΔH_i° , the four Adair constants, ΔH° and the oxygen partial pressure of the hemoglobin sample, p_{O_2} . The first nine parameters may be found in the literature¹⁴, since they are experimentally determined quantities. The comparative (χ) and relative (ρ) efficiencies, defined for conditions (i) and (ii), also apply to hemoglobin, according to the equations:

$$\chi = \frac{\Delta H^{\text{Hb}}}{[\text{S}]_{\text{T}} \Delta Y^{\text{Hb}}} - \frac{\Delta H^{\text{Mb}}}{[\text{S}]_{\text{T}} \Delta Y^{\text{Mb}}} \quad (18)$$

$$\rho = \frac{\Delta Y^{\text{Hb}} - \Delta Y^{\text{Mb}}}{\Delta Y^{\text{Hb}}} \quad (19)$$

χ and ρ also obey equation (13), as well as the following equations, analogous to (15) and (16):

$$\chi = \frac{\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}}{[\text{S}]_{\text{T}} \Delta Y^{\text{Hb}}} \quad (20)$$

$$\rho = - \frac{\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}}{[\text{S}]_{\text{T}} \Delta Y^{\text{Hb}} \Delta H^{\circ}} \quad (21)$$

4 RESULTS AND DISCUSSION

Reliable experimental values of the ΔH_i° s are scarce in the literature. We took the four ΔH_i values and the four Adair constants from the work of Imai¹⁴ and ΔH° , the standard enthalpy of oxygenation of myoglobin from Antonini and Brunori¹⁵. Imai has analysed six different experimental conditions, but we will restrict ourselves to four of his cases (condition set numbers 3,4,5 and 6).

We have plotted in Figures 1 and 2 the variation of χ and of $\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}$ with respect to both the oxygen partial pressure of the hemoglobin's sample and the extent of oxygen release. In all calculations we have assumed that the initial value of the fractional saturation (high p_{O_2}) is 1, so that $\Delta Y^{\text{Hb}} = Y^{\text{Hb}} - 1$. No graph of ρ is shown since it is proportional to χ according

to equation (13). It is seen that the experimental conditions have a great effect upon the analysed functions. In particular, Imai's case 3 shows a minor deviation from the behaviour predicted by the dimer model in the intermediate and high oxygen partial pressure ranges, where χ becomes negative and $\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}$ positive.

Imai's cases 5 and 6, which represent hemoglobin, respectively, in the presence of DPG and IHP, are the ones that markedly follow the dimer model. Both effectors bind to the $\beta\beta$ interface, increasing the functional non-equivalence of the α and β chains and amplifying, in this way, the functions χ and $\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}$; it is known¹⁶ that IHP (case 6) binds much more strongly than DPG. This is shown in Figures 1 and 2, where we see that χ is the highest and $\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}$ is the lowest in the curves of case 6.

Imai's cases 3 and 4, on the other hand, as shown in Figures 1 and 2, behave distinctly from cases 5 and 6. Thus, in figures 1(b) and 2(b) we see that, up to the very low range of liberated oxygen, case 3 is thermodynamically more efficient than case 4; this is to be attributed to the extent and heat of proton binding, for in case 3, the hemoglobin being in an alkaline medium, binds protons, when delivering oxygen, to a greater extent than case 4. Since the heat of proton binding is comparable to the heat of oxygen binding¹⁷ (and figures 1 and 2 are a composite of oxygen and ion exchanges) it follows that the heat requirement for oxygen release is somewhat compensated by the heat of proton binding.

A similar comparison between figures 2(a) and 2(b) shows

that the function $\Delta H^{\text{Hb}} - \Delta H^{\text{Mb}}$ (condition (iii)), has an approximately linear decrease with respect to the extent of oxygen release whereas it decreases sigmoidally with increasing oxygen partial pressures. This effect has important consequences, namely, that hemoglobin's cooperativity shows itself not only with respect to oxygen transfer but also to heat exchange, and that hemoglobin, starting from 100% fractional saturation, becomes, from an energetic point of view, more and more advantageous than myoglobin (representing hemoglobin's ancestor) as the the oxygen concentration decreases.

Figures 1 and 2 are related by equation (20). Case 5, for instance, is, in figure 2(b), a straight line pointing to zero (except for a small deviation close to zero liberated O_2 which cannot be seen in the chosen scale). From this linear character one should expect, through equation (20), that χ remains approximately constant with respect to the extent of oxygen; this in fact, shows in figure 1(b) up to 0.0002 moles of liberated oxygen. The same applies to cases 3 and 4. The curve corresponding to case 6 (Figure 2(b) does not point to zero, hence the decay of case 6 curve in Figure 1(b).

It is seen that the predictions of the simple dimer model are confirmed to a large extent by the hemoglobin calculations. It seems an inescapable conclusion that the differentiation of the α and β chains give rise to an improved mechanism of energy utilization, relatively to the ancestral monomeric species. For many years now, starting with the work of Benesch and Benesch^{18,19,20} it is known that human β_4 hemoglobin is not allosteric. However, there is no known physical reason

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why a homotetrameric protein cannot bind O_2 cooperatively, and, on the other hand, $\alpha_2\epsilon_2$ hemoglobins are known²¹ which also are not cooperative. What we have shown is that, starting from a monomeric oxygen carrier, the assembly of tetrameric species is advantageous if one uses two pairs of different chains.

We should like to suggest that the same general principle work for non-human hemoglobins, as well as for other allosteric systems, and we predict that an analysis of the literature data on allosteric enzymatic systems will show further examples of this behaviour.

FIGURE CAPTIONS

Fig. 1(a) Variation of Comparative Efficiency (χ) as a function of oxygen partial pressure.

- . — . — Imai's case 3 (0.1MCl^- , $\text{PH} = 7.4$);
 Imai's case 4 (0.1MCl^- , $\text{ph} = 6.5$);
 ————— Imai's case 5 (0.1MCl^- , 2m MDPG , $\text{pH} = 7.4$);
 ----- Imai's case 6 (0.1MCl^- , 2m M IHP , $\text{pH} = 7.4$);

Fig. 1(b) Variation of Comparative Efficiency (χ) as a function of liberated O_2 . Curves as in Fig. 1(a).

Fig. 2(a) Variation of $\Delta\text{H}^{\text{Hb}} - \Delta\text{H}^{\text{Mb}}$ as a function of oxygen partial pressure. Curves as in Fig. 1(a).

Fig. 2(b) Variation of $\Delta\text{H}^{\text{Hb}} - \Delta\text{H}^{\text{Mb}}$ as a function of liberated O_2 . Curves as in Fig. 1(a).

FIG. 1(a)

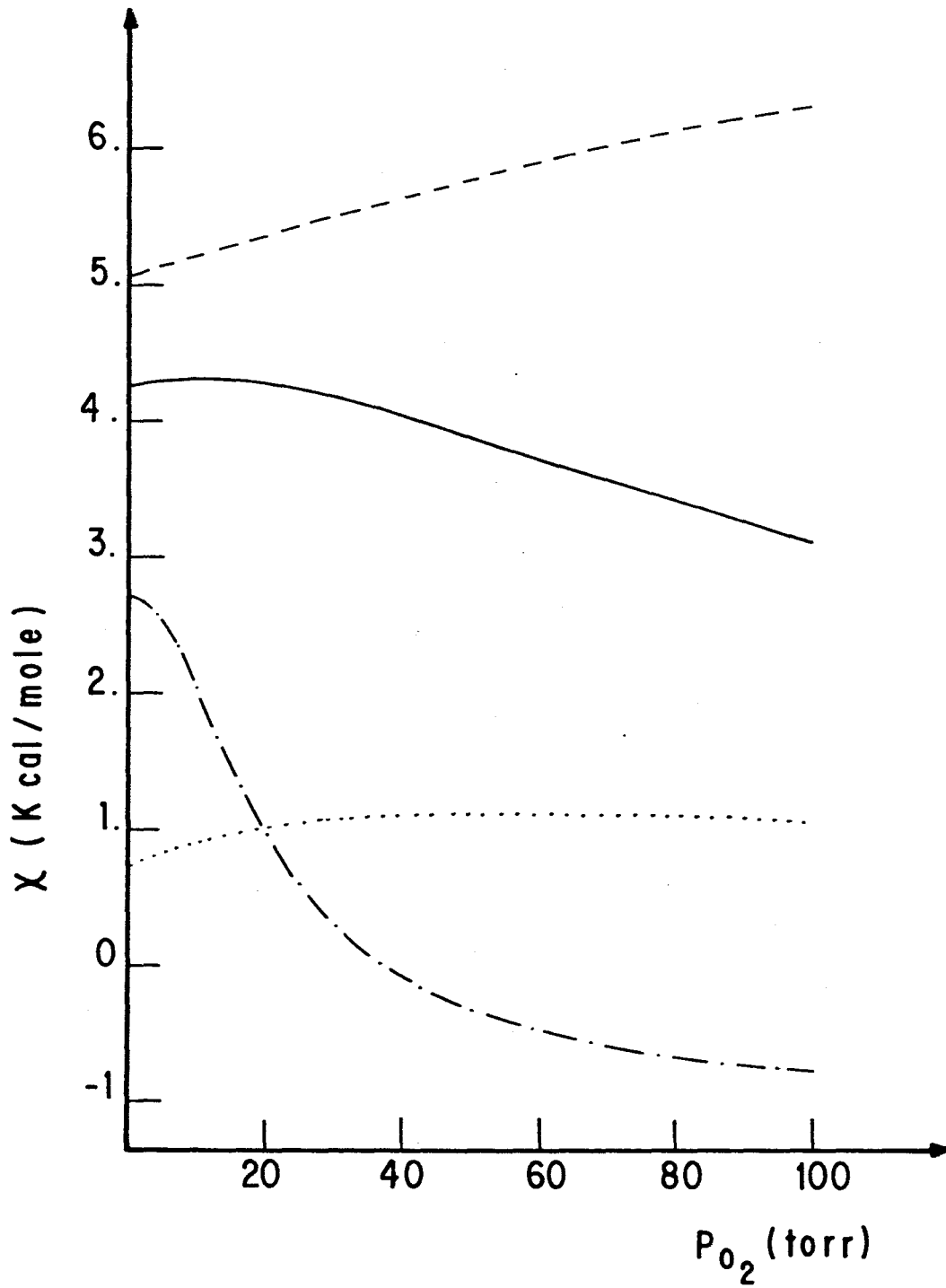


FIG. 1(b)

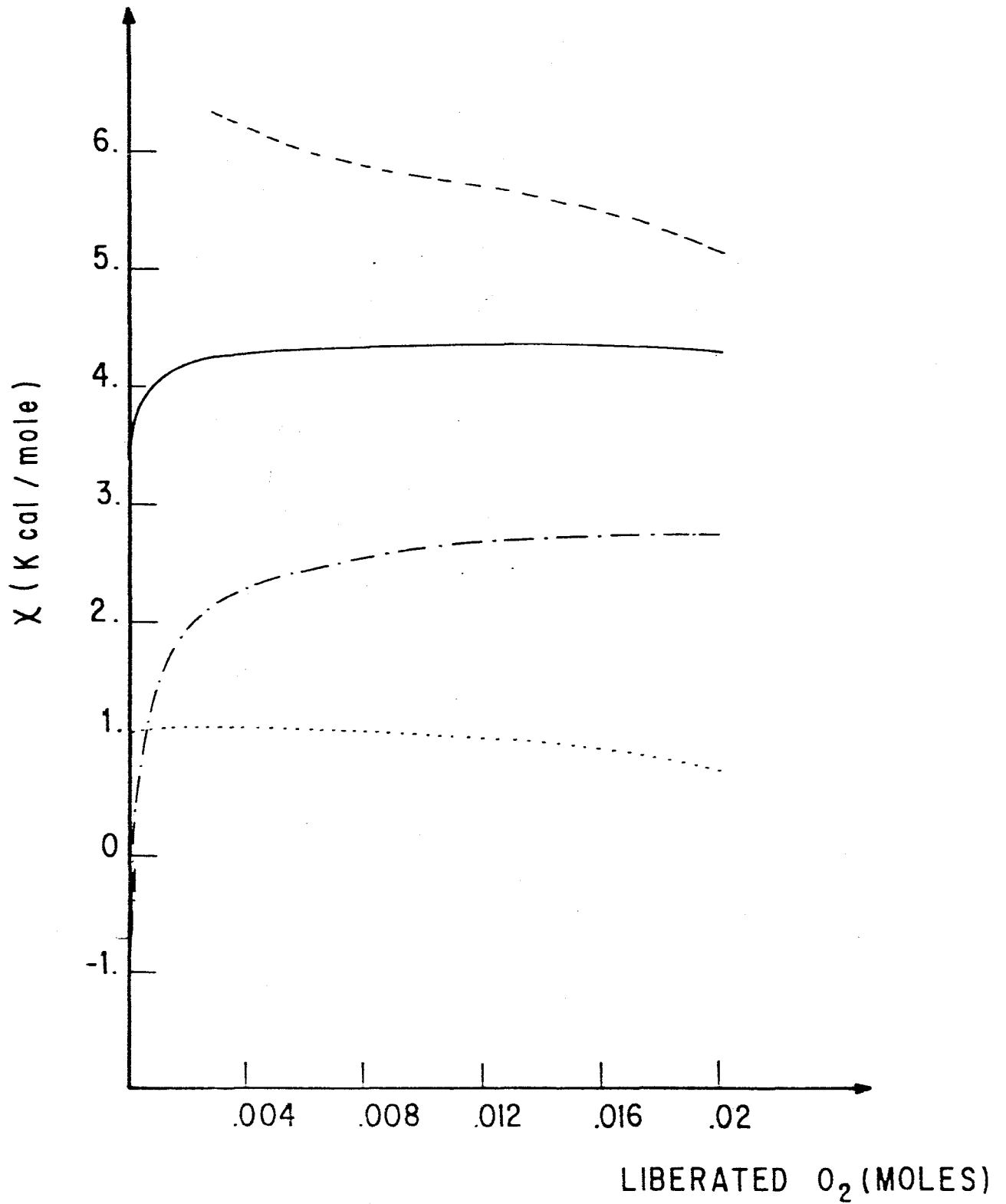


FIG. 2 (a)

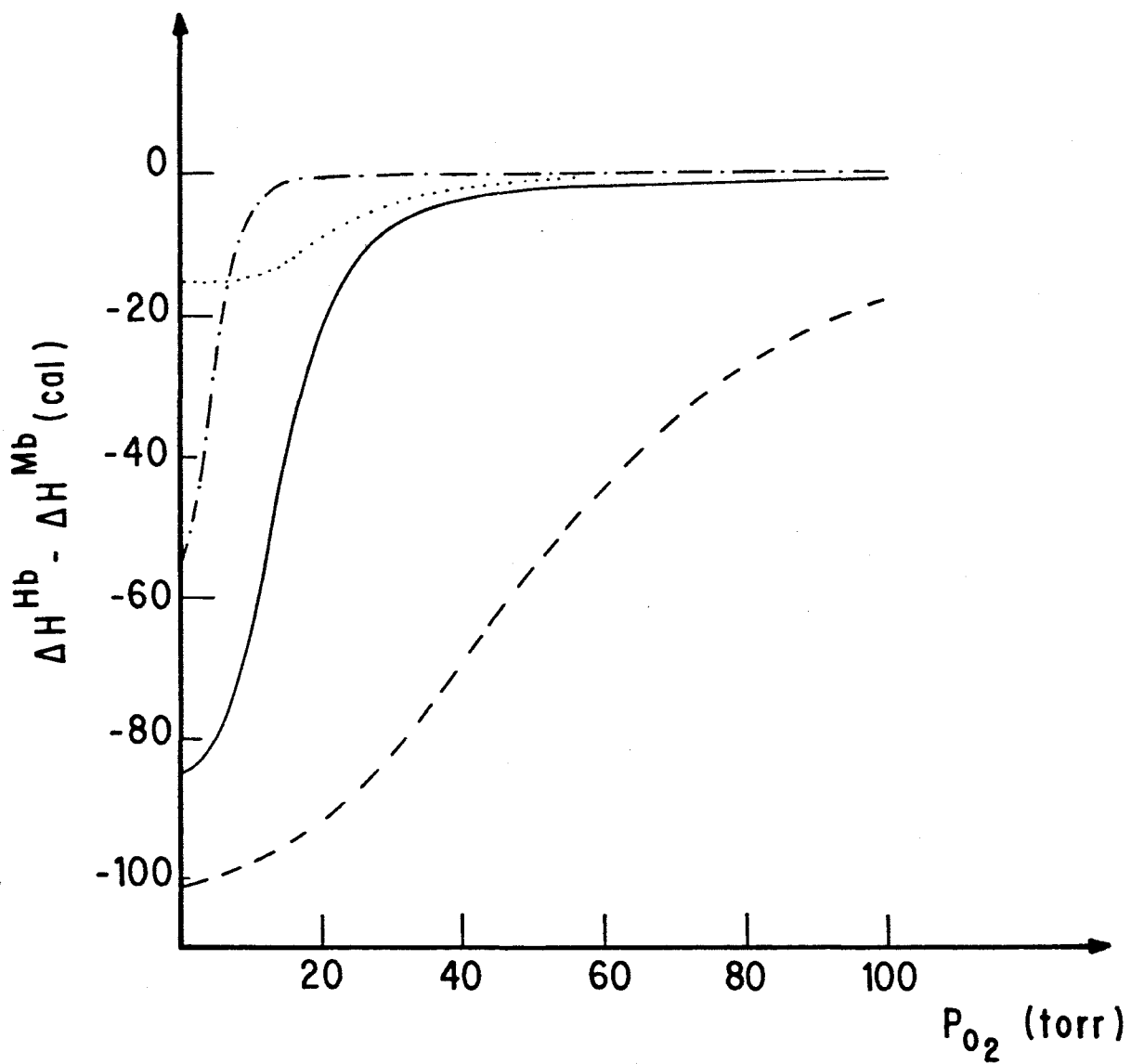
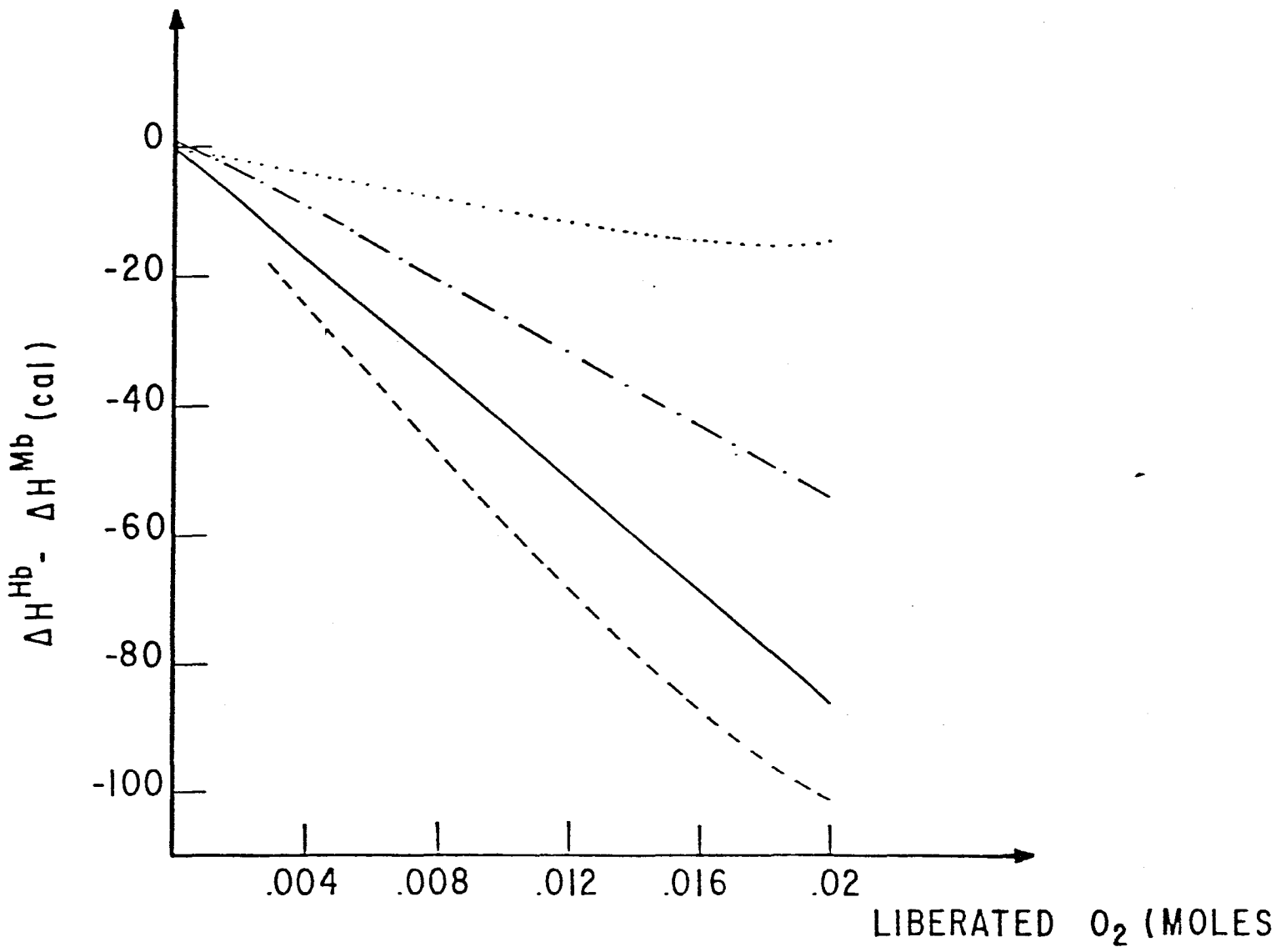


FIG. 2 (b)



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