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CALCULUS OF THE SITE SPECIFIC OXYGEN BINDING CONSTANTS OF HEMOGLOBIN

by

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ABSTRACT

The site specific binding constants of the hemoglobin-dioxygen equilibria were calculated with the aid of two approximations based in distinct, though related, features of the hemoglobin's problem. The first approximation (AI) is based on the well stablished proeminency of the role played by the $\alpha_I\beta_{II}$ interfaces (Perutz, 1970) in the mechanism of cooperativity. The second approximation (AII) is based in the selective character of the binding of some organic phosphates to the ββ interface (Benesch, Benesch and Enoki, 1968; Arnone and Perutz, 1974). A comparison between the two approximations showed that, except for the constraining of the β sites binding constants in AI, the two approximations give similar results. Also, the present calculations agree with known facts, such as the preferential binding of the α sites (Lindstron and Ho, 1972; Johnson and Ho, 1974; Asakura and Pui-Wah, 1978) and the quaternary enhancement (Mills ans Ackers, 1979).

Key-words: Hemoglobin; Dioxygen binding constants; Non-equivalent sites.

1 INTRODUCTION

Recently the possible role of the functional non-equivalence of binding sites in hemoglobins has been discussed. On one hand the non-equivalence guarantees the well known assymmetry of the saturation curve, which may be important for resting animals (Peller, 1982; Weber, 1982). The present authors, on the other hand, have discussed (Ferreira and Jacchieri, 1984), the effect of the functional non-equivalence of hemoglobin's chains on the thermal invariance of its cooperative behaviour. These analyses brought into view the importance of knowing the values of the α and β chains binding constants in the various stages of oxygenation.

As shown in Figure 1, the inclusion of chain hetereogeneity in the equilibrium description leads to a complicated pattern with sixteen binding constants. Even considering energy conservation constraints of oxygenation states connected by different paths (such as $k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} = k_\alpha k_{\alpha_I\beta_I} k_{\alpha_I\beta_I\alpha_{II}}$), there remain nine site specific constants which one may choose to be $k_\alpha, k_{\alpha\alpha}, k_\beta, k_{\beta\beta}$, $k_{\alpha_I\beta_I}, k_{\alpha_I\beta_{II}}, k_{\alpha\alpha\beta}$, $k_{\beta_I\alpha_I\beta_{II}}$ and $k_{\alpha\alpha\beta\beta}$. These constants are related to the four extrinsic ones of the well known Adair scheme (Adair, 1925) by the equations:

$$K_1 = 2k_\alpha + 2k_\beta \tag{1}$$

$$K_2 = \frac{k_\alpha k_{\alpha\alpha} + k_\beta k_{\beta\beta} + 2k_\alpha k_{\alpha_I\beta_I} + 2k_\alpha k_{\alpha_I\beta_{II}}}{2k_\alpha + 2k_\beta} \tag{2}$$

$$K_3 = \frac{2k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} + 2k_\alpha k_{\alpha_I\beta_I} k_{\beta_I\alpha_I\beta_{II}}}{k_\alpha k_{\alpha\alpha} + k_\beta k_{\beta\beta} + 2k_\alpha k_{\alpha_I\beta_I} + 2k_\alpha k_{\alpha_I\beta_{II}}} \tag{3}$$

$$K_4 = \frac{k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} k_{\alpha\alpha\beta\beta}}{2k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} + 2k_\alpha k_{\alpha_I\beta_I} k_{\beta_I\alpha_I\beta_{II}}} \qquad (4)$$

The fractional saturation curve given by Adair's equation is therefore a composite of nine site specific constants. Unless further measurements which discriminate between the α and β chains are available, approximations must be made to solve the above system of four equations and nine unknowns.

Experimental discriminating information in fact exist to some extent. The preferential binding of the α chains was studied with NMR (Lindstrom and Ho, 1972; Johnson and Ho, 1974) and spin label (Asakura and Pui-wah,1978) experiments. A recent structure determination (Brzozowski et al. 1984) with X-ray diffraction also showed that hemoglobin in the first stages of oxygenation is mainly α occupied. These results are usefull in providing additional insight of the chain's differentiation. In fact, we will show elsewhere (Jacchieri, 1985) how the necessary information can be obtained from EPR measurements.

Even without making use of direct measurements of the site's relative affinities one can profit from the fact that the oxygen saturation curves reflect the chain hetereogeneity. This is the basis of the first of a set of two approximations which will be referred to as AI and AII. Also there are heterotropic effectors such as DPG and IHP which bind selectively to the ββ interface (Benesch, Benesch and Enoki 1968; Arnone and Perutz 1974), and by the changes they cause in the Adair constants it should be possible to derive expressions for the site specific constants.

2 APPROXIMATION I

Equations (1) to (4) apply to any case in which two pairs of non-equivalent sites bind reversibly to one ligand. To obtain equations more specifically related to the hemoglobin problem, as well as to reduce the number of unknowns, we have made use of known proposals about the roles played by the $\alpha\alpha$, $\beta\beta$, $\alpha_I\beta_I$ and $\alpha_I\beta_{II}$ interfaces.

The work of Perutz (1970) gives strong evidences that the cooperative interactions occur mainly through the $\alpha_I\beta_{II}$ interfaces, whereas the $\alpha_I\beta_I$ interfaces remain almost unchanged in the course of oxygenation. It also seems that the number and kind of weak bonds broken in the $\alpha\alpha$ interface when one α site is occupied does not depend on the occupancy of the another one. Although the equivalence of α sites may be assumed, the second β site occupation turns out to be non equivalent to the first one as a consequence of the $\beta\beta$ pocket opening (Baldwin, 1975). This feature although not explicitly considered in AI, is taken in account in AII.

This discussion suggest that we can make two well fundamented or "strong" approximations:

$$k_\alpha = k_{\alpha\alpha} \tag{5}$$

$$k_\beta = k_{\alpha_I\beta_I} \tag{6}$$

The original set of nine unknowns is now reduced to seven, namely $k_\alpha, k_\beta, k_{\beta\beta}, k_{\alpha_I\beta_{II}}, k_{\alpha\alpha\beta}, k_{\beta_I\alpha_I\beta_{II}}$ and $k_{\alpha\alpha\beta\beta}$. At this stage, to further reduce the number of unknowns it is necessary to

make more drastic approximations. We have chose the following ones, called "weak" approximations:

$$k_{\beta} = k_{\beta\beta} \tag{7}$$

$$k_{\alpha\alpha\beta} = k_{\alpha_I\beta_{II}} = k'_{\alpha\beta} \tag{8}$$

$$k_{\beta_I\alpha_I\beta_{II}} = k_{\alpha\alpha\beta\beta} = k''_{\alpha\beta} \tag{9}$$

Equations (8) and (9) are based in the idea that the allosteric interaction is independent of the previous occupation of one α site (eq. (8) but depends on the previous occupation of one β site (eq. (9). As for equation (7) its consequences will be discussed later. In this way we end up with the four unknowns $k_{\alpha}, k_{\beta}, k_{\alpha\beta}$ and $k''_{\alpha\beta}$. By using the products $c_1 = k_1$, $c_2 = k_1k_2$, etc..., we arrive at the set:

$$c_1 = 2k_{\alpha} + 2k_{\beta} \tag{10}$$

$$c_2 = (k_{\alpha}+k_{\beta})^2 + 2k_{\alpha}k'_{\alpha\beta} \tag{11}$$

$$c_3 = 2k_{\alpha}^2k'_{\alpha\beta} + 2k_{\alpha}k_{\beta}k''_{\alpha\beta} \tag{12}$$

$$c_4 = k^2k'_{\alpha\beta}k''_{\alpha\beta} \tag{13}$$

whose solution is :

$$k_\beta = \frac{C_3 - \frac{C_1}{2}\left[C_2 - \frac{C_1^2}{4}\right]}{\frac{4C_4}{C_2 - \frac{C_1^2}{4}} - C_2 + \frac{C_1^2}{4}} \tag{14}$$

$$k_\alpha = \frac{C_1}{2} - k_\beta \tag{15}$$

$$k_{\alpha\beta}' = \frac{C_2 - \frac{C_1^2}{4}}{C_1 - 2k_\beta} \tag{16}$$

$$k''_{\alpha\beta} = \frac{C_4}{k_\alpha^2 k'_{\alpha\beta}} \tag{17}$$

This set of equations ((14) to (17)) constitutes Approximation I.

3 APPROXIMATION II

This approximation makes use of the selective character of the binding of some organic phosphates to the ββ interface (Benesch, Benesch and Enoki, 1968; Arnone and Perutz, 1974). We assume that the α site binding constants are not affected by the binding of DPG (or IHP). The Adair constants vary in the presence of increasing concentration of DPG due to changes in the β site binding constants.

The simultaneous equilibria of one protein with two or more ligands is treated by the Linked Functions Theory (Wyman, 1964). Thus, the DPG binding constants M_0 and M_1, related to the equations:

$$Hb + DPG \overset{M_0}{\rightleftharpoons} HbDPG \tag{18}$$

$$Hb(O_2) + DPG \overset{M_1}{\rightleftharpoons} Hb(O_2)DPG \tag{19}$$

are determined by the changes of the Adair constants with the DPG concentration (Tyuma, Imai and Shimizu, 1973; Imaizumi, Imai and Tyuma, 1979). The site specific constants M_α and M_β, which refer to the reactions :

$$\alpha O_2\alpha\beta\beta + DPG \overset{M_\alpha}{\rightleftharpoons} \alpha O_2\alpha\beta\beta DPG \tag{20}$$

$$\alpha\alpha\beta O_2\beta + DPG \overset{M_\beta}{\rightleftharpoons} \alpha\alpha\beta O_2\beta DPG \tag{21}$$

were not evaluated as far as we known but are required for our approximation.

We suppose that the DPG molecules bound to the ββ pocket's are displaced after the occupation of the first β site. This justifies the assumption that $k_{\beta\beta}$ is equal to $\bar{k}_\beta$ (barred k's will indicate zero DPG concentration), that is, after the oxygenation of the first β site, the affinity of the other one becomes independent of the DPG concentration and equal to the value it should have in the absence of DPG.

A simple reasoning shows that

$$M_1 = \frac{[HbO_2DPG]}{[HbO_2][DPG]} = \frac{\{[\alpha O_2\alpha\beta\beta DPG] + [\alpha\alpha\beta O_2\beta DPG]\}}{\{[\alpha O_2\alpha\beta\beta] + [\alpha\alpha\beta O_2\beta]\}[DPG]} \tag{22}$$

By considering the equilibria (21) and (23):

$$\alpha\alpha\beta\beta + O_2 \overset{2\bar{k}_\beta}{\rightleftharpoons} \alpha\alpha\beta O_2\beta \tag{23}$$

we conclude that $[\alpha\alpha\beta O_2\beta DPG] = 2\bar{k}_\beta M_\beta [Hb][O_2][DPG]$, Similarly, we have $[\alpha O_2\alpha\beta\beta DPG] = 2k_\alpha M_\alpha [Hb][O_2][DPG]$, so that

$$M_1 = \frac{2k_\alpha M_\alpha + 2\bar{k}_\beta M_\beta}{2k_\alpha + 2\bar{k}_\beta} = \frac{2k_\alpha M_\alpha + 2\bar{k}_\beta M_\beta}{\bar{k}_1} \tag{24}$$

An overwhelming number of evidences (Lindstrom and Ho, 1972; Johnson and Ho, 1974, Asakura and Pui-Wah, 1978; Brzozowski et al. 1984) in favour of the α sites preferential binding suggests that $k_\beta << k_\alpha$. Also, the fact that DPG competes with dioxygen for the occupation of the β sites (Baldwin, 1975) comes in favour of the inequality $M_\beta < M_\alpha$. These cumulative trends enable us to discard $2\bar{k}_\beta M_\beta$ in equation (24). The remaining unknown, M_α, may be found out by the already made hypothesis that α-sites oxygen binding constants are unaffected by the presence of DPG molecules in the $\beta\beta$ pockets, which is, to say that reactions (18) and (20) are equivalent, or $M_\alpha = M_o$. We have now one equation for k_α

$$k_\alpha = \frac{\bar{k}_1 M_1}{2M_o} \tag{25}$$

Inserting the above discussed approximations $\bar{k}_\beta = k_{\beta\beta}$ and equation (25) plus the strong approximation of AI in the Adair constants equations (1) to (4) we are able to calculate $\bar{k}_\beta = k_{\beta\beta}$, k_β, $k_{\alpha_I\beta_{II}}$ and $k_{\alpha\alpha\beta}k_{\alpha\alpha\beta\beta}$ through the relations

$$k_{\beta\beta} = \bar{k}_\beta = \frac{\bar{k}_1 (M_o - M_1)}{2M_o} \tag{28}$$

$$k_\beta = \frac{k_1 M_o - \bar{k}_1 M_1}{2M_o} \tag{29}$$

$$k_{\alpha_I\beta_{II}} = \frac{C_2 - k_\alpha^2 - 2k_\alpha k_\beta - k_\beta \bar{k}_\beta}{2k_\alpha} \tag{30}$$

$$k_{\alpha\alpha\beta}k_{\alpha\alpha\beta\beta} = \frac{C_4}{k_\alpha^2} \tag{31}$$

it being that the products $c_1,\ldots,c_4$ were defined in AI.

It is interesting to note that since:

$$\frac{k_\alpha}{\bar{k}_\beta} = \frac{M_1}{M_o - M_1} \tag{32}$$

the ratio $k_\alpha/\bar{k}_\beta$ depends only on the DPG binding constants M_o and M_1.

4 RESULTS AND DISCUSSION

Tyuma, Imai and Shimizu, 1973 (TIS) have determined the four Adair constants of oxygen binding to hemoglobin in various DPG concentrations as well as the DPG binding constants to the Hb and $Hb(O_2)$ species. Their results enable a simultaneous application of AI and AII and the subsequent comparison of the two approximations.

In Table 1 we show the values, expressed in terms of free energies of binding, obtained from the application of AI to the TIS data. Although AI does not take explicitelly in account the selectivity of DPG binding, it does reflect this feature. A comparison between the first and second rows of Table 1 shows that ΔG_α^0 remains almost unaffected by the presence of DPG,

whereas ΔG^0_β changes significatively, $\Delta 6^0_\beta$", on the other hand is also almost insensitive to changes in DPG concentration. This in accordance with the fact, which may be derived from the small relative value of the DPG binding constant to $(Hb(O_2)_4$, that in the last stages of oxygenation most DPG molecules were already displaced from the ββ pockets.

An important consequence of the "weak" approximations of AI ($k_\beta = k_{\beta\beta}$ particularly) is that the resulting value of $\Delta G^0_{\beta\beta\alpha}$ is of the order of - 11 kcal/cal, an exceedingly high binding constant. Nonetheless, the values of $\Delta G^{0}_{\alpha\beta}{}'' = \Delta G^0_{\alpha\alpha\beta\beta}$ are slightly more negative than the free energie of binding of free β chains. This is in accordance with the quaternary enhancement effect (Mills and Ackers, 1979) that makes the last O_2 binding constant of assembled chains larger than those of free ones.

The application of AII to the same data is shown on Table 2. In this case the selectivity of DPG binding is an imposed condition, hence the constancy of ΔG^0_α which is, nonetheless, similar to its values shown on Table 1. Since AII does not have the k_β constraining approximation, $k_\beta = k_{\beta\beta}$, the values of ΔG^0_β turn-out to be closer to ΔG^0_α than in AI, as can be seen by comparing $\Delta G^0_\alpha - \Delta G^0_\beta$ in Tables 1 and 2. The other features of AII are similar to AI; thus, $\Delta G^0_{\alpha_I\beta_{II}}$ ($\Delta G^{0'}_{\alpha\beta}$ in AI) and $\Delta G^0_{\alpha\alpha\beta} + \Delta G^0_{\alpha\alpha\beta\beta}$ ($\Delta G^{0'}_{\alpha\beta} + \Delta G^{0}_{\alpha\beta}{}''$ in AI) are almost equal to the values obtained with AI.

We have discussed elsewhere (Ferreira and Jacchieri, 1984) the results obtained with the application of AI to data measured in various temperatures (Imai; 1979). In that case it is possible to calculate enthalpies and entropies of oxygen binding

in additon to free energies.

The importance of precise determinations of hemoglobin's site-specific oxygen binding constants for the understanding of the mechanism of cooperativity was recently realized. Future improvements in this subject will come from combined efforts of both theory and experiment.

CAPTIONS

Figure 1 - Diagram showing the site specific free-energies of dioxygen binding.

Table 1 - Results obtained from Approximation I (see text) applied to data from Tyuma, Imai and Shimizu (1973). In the following cases the Adair constants were corrected within the experimental error:

a) [DPG] = 0.5mM; $k_1 = 0.0147 - 0.0013$; $k_2 = 0.0213 - 0.0065$; $k_3 = 0.0195 + 0.0089$

b) [DPG] = 1.0mM; $k_1 = 0.0139 - 0.0010$; $k_2 = 0.00719 - 0-00405$.

Table 2 - Results obtained from Approximation II (see text) applied to data from Tyuma, Imai and Shimizy (1973). In the following casrs the Adair constants were corrected within the experimental error.

[DPG] 1.0mM: $k_1 = 0.0139 + 0.0005$; $k_2 = 0.00719 + 0.00405$

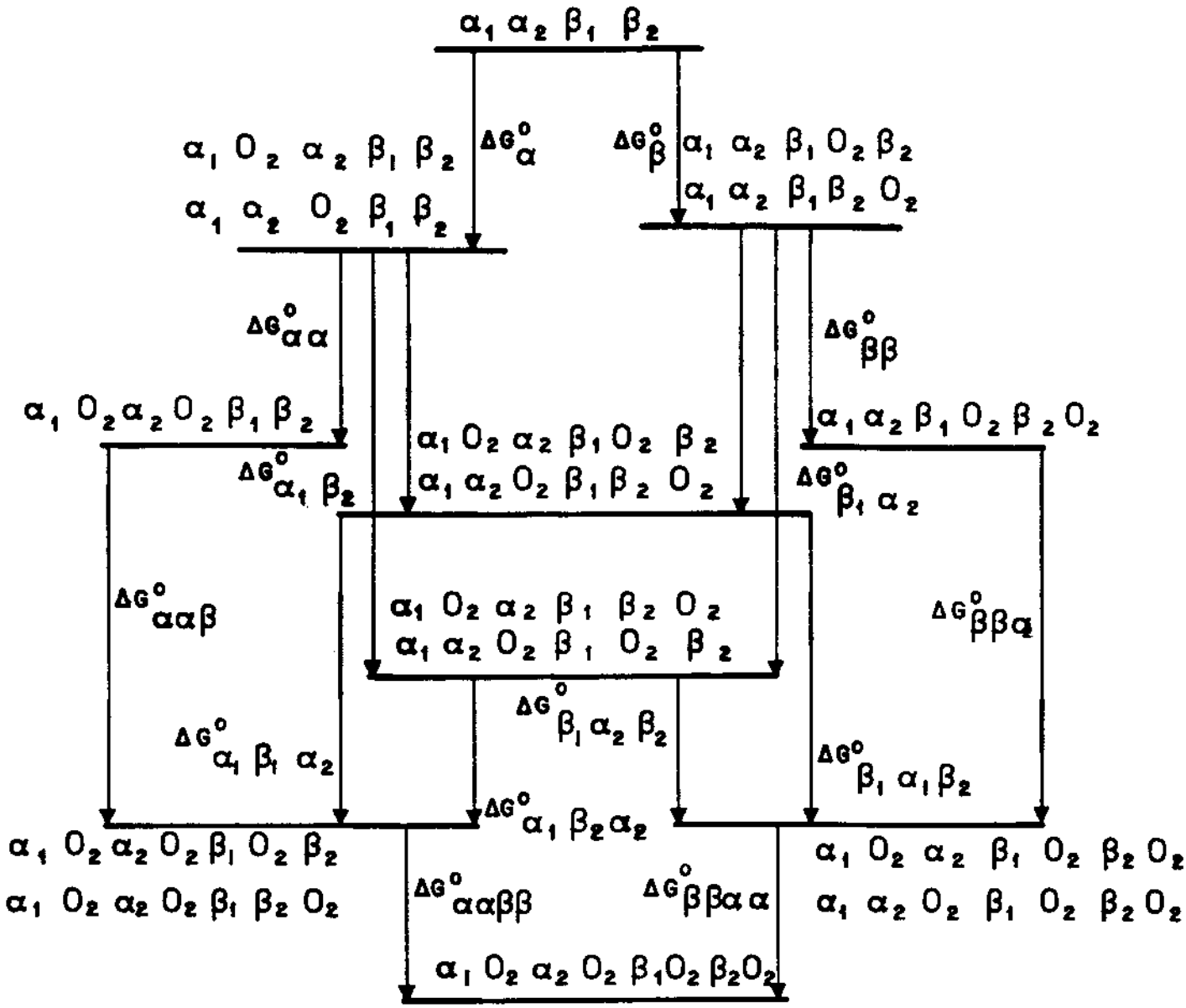
$\alpha_1\,\alpha_2\,\beta_1\,\beta_2$
ΔG°_α
ΔG°_β
$\alpha_1\,O_2\,\alpha_2\,\beta_1\,\beta_2$
$\alpha_1\,\alpha_2\,O_2\,\beta_1\,\beta_2$
$\alpha_1\,\alpha_2\,\beta_1\,O_2\,\beta_2$
$\alpha_1\,\alpha_2\,\beta_1\,\beta_2\,O_2$
$\Delta G^\circ_{\alpha\alpha}$
$\Delta G^\circ_{\beta\beta}$
$\alpha_1\,O_2\,\alpha_2\,O_2\,\beta_1\,\beta_2$
$\alpha_1\,O_2\,\alpha_2\,\beta_1\,O_2\,\beta_2$
$\alpha_1\,\alpha_2\,O_2\,\beta_1\,\beta_2\,O_2$
$\alpha_1\,\alpha_2\,\beta_1\,O_2\,\beta_2\,O_2$
$\Delta G^\circ_{\alpha_1\,\beta_2}$
$\Delta G^\circ_{\beta_1\,\alpha_2}$
$\Delta G^\circ_{\alpha\alpha\beta}$
$\Delta G^\circ_{\beta\beta\alpha}$
$\alpha_1\,O_2\,\alpha_2\,\beta_1\,\beta_2\,O_2$
$\alpha_1\,\alpha_2\,O_2\,\beta_1\,O_2\,\beta_2$
$\Delta G^\circ_{\beta_1\,\alpha_2\,\beta_2}$
$\Delta G^\circ_{\alpha_1\,\beta_1\,\alpha_2}$
$\Delta G^\circ_{\beta_1\,\alpha_1\,\beta_2}$
$\Delta G^\circ_{\alpha_1\,\beta_2\,\alpha_2}$
$\alpha_1\,O_2\,\alpha_2\,O_2\,\beta_1\,O_2\,\beta_2$
$\alpha_1\,O_2\,\alpha_2\,\beta_1\,O_2\,\beta_2\,O_2$
$\alpha_1\,O_2\,\alpha_2\,O_2\,\beta_1\,\beta_2\,O_2$
$\alpha_1\,\alpha_2\,O_2\,\beta_1\,O_2\,\beta_2\,O_2$
$\Delta G^\circ_{\alpha\alpha\beta\beta}$
$\Delta G^\circ_{\beta\beta\alpha\alpha}$
$\alpha_1\,O_2\,\alpha_2\,O_2\,\beta_1\,O_2\,\beta_2\,O_2$

TABLE 1

[DPG] mM	ΔG^0_α Kcal/mol	ΔG^0_β Kcal/mol	$\Delta G^{0'}_{\alpha\beta}$ Kcal/mol	$\Delta G^{0''}_{\alpha\beta}$ Kcal/mol	$\Delta G^0_\alpha - \Delta G^0_\beta$ Kcal/mol	$G^{0'}_{\alpha\beta}$ Kcal/mol	$G^{0''}_{\alpha\beta}$ Kcal/mol	$G^0_{34/12}$ Kcal/mol
0,0	-6,24±0,05	-3,41±0,53	-6,60±0,17	-9,06±0.27	-2,83±0,54	-3,19±0,56	-5,65±0,60	-3,48±0,52
0,2	-6,13±0,04	-2,17±0,55	-5,38±0,42	-9,32±045	-3,96±0,55	-3,21±0,69	-7,35±0,71	-4,23±0,53
0,5	-5,89±0,11	-1,72±1,02	-5,24±0,71	-9,27±0,76	-4,17±0,10	-3,52±1,2	-7,55±1,3	-3,57±0,90
1,0	-5,88±0,09	-1,53±0,94	-4,76±0,86	-9,66±0,88	-4,34±0,95	-3,23±1,27	-8,12±1,29	-4,70±1,06

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TABLE 2

[DPG] mM	ΔG^0_α Kcal/mol	ΔG^0_β ([DPG]=0) Kcal/mol	ΔG^0_β Kcal/mol	$\Delta G^0_\alpha - \Delta G^0_\beta$ Kcal/mol	$\Delta G^0_{\alpha_I\beta_{II}}$ Kcal/mol	$\Delta G^0_{\alpha\alpha\beta} + \Delta G^0_{\alpha\alpha\beta\beta}$ Kcal/mol
0,0	-5,94±0,1	-5,74±0,18	-5,74±0,18	-0,20±0,28	-6,93±0,20	-16,33±0,25
0,2	-5,94±0,1	-5,74±0,18	-5,43±0,24	-0,51±0,34	-5,54±0,50	-15,35±0,26
0,5	-5,94±0,1	-5,74±0,18	-4,16±0,96	-1,78±1,06	-5,67±0,33	-14,55±0,32
1,0	-5,94±0,1	-5,74±0,18	-3,82±1,27	-2,12±1,37	-4,45±1,20	-14,42±0,44

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