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Free Energy Couplings between Ligand Binding and Subunit Association in Hemoglobin Are of First Order

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ABSTRACT: The calculations presented in a recent paper [Johnson, M. L. (1986) *Biochemistry 25*, 791–797] to the effect that the free energy couplings between oxygen binding and subunit association in hemoglobin A can be of either first or second order are examined. The fitting of the experimental data to a system with second-order couplings carried out by Johnson belongs to a tetramer in which, in contradistinction to hemoglobin A, oxygen binding promotes subunit association.

The analysis of the relations between the binding of ligands to oligomeric proteins and the binding of the monomers to each other within the aggregate may be done by postulating the existence of standard free energy couplings between the two kinds of equilibria involved: protein–protein and protein–ligand associations (Weber, 1972).

The total standard free energy of binding of 4 mol of oxygen to four noninteracting, i.e., independent, subunits is that of the reaction $4M + 4X \rightarrow 4MX$, where M symbolizes the subunit and X the ligand, oxygen in this case. I designate this total free energy as $dG°(\text{sub})$. The standard free energy of binding of 4 mol of oxygen to the tetramer is that of the reaction $T + 4X \rightarrow TX_4$, and I designate this as $dG°(\text{tet})$. In the absence of any coupling between the free energy of subunit interaction and that of oxygen binding, we must have

$$dG°(\text{sub}) = dG°(\text{tet}) \qquad (1)$$

If these two free energies differ from each other, there are two possibilities. The first case is

$$|dG°(\text{sub})| > |dG°(\text{tet})| \qquad (2)$$

where the quantities between bars denote absolute values. In this case, the defect in the free energy of binding by the tetramer is the energy employed in overcoming binding constraints that reside at the boundary between subunits. As a consequence, the binding of the ligand promotes oligomer dissociation. The second case is

$$|dG°(\text{sub})| < |dG°(\text{tet})| \qquad (3)$$

This case is the opposite of the preceding one. The free energy of ligand binding by the tetramer is augmented by a contribution from increased subunit interaction: Ligand binding promotes association.

Any model proposed for the coupling of oxygen binding to subunit interaction in hemoglobin A should be one that obeys relation 2 as it has been known, for at least 15 years, that oxygen binding promotes subunit dissociation.

I have introduced the distinction between free energy couplings of different orders that I designate as first order, second order, etc. (Weber, 1982). Further, I have concluded (Weber, 1984) that the coupling between the free energy of subunit interaction and that of oxygen binding in hemoglobin A is, unequivocally, of first order.

In a recent paper Johnson (1986) purports to show that second-order couplings, as well as those of first order, can account for the experimental values of the free energies of the four successive additions of oxygen to hemoglobin. A detailed analysis of the orders of coupling is not necessary for our present purpose, and I refer the reader to the original papers (Weber, 1982, 1984) for further information. The calculations that are supposed to demonstrate that both first- and second-order couplings apply to hemoglobin A equally well are shown in Tables III and IV of Johnson's paper. The procedure used in the calculations is essentially that employed by Weber (1982), with the difference that in the calculations by Johnson the free energies of binding of O_2 by the isolated chains are treated as further adjustable parameters while Weber used the experimentally determined values. This difference is unimportant as the free energy conservation relations 1–3 apply in either case. Johnson subjected two sets of experimental data to similar analysis, and as they yield completely concordant results, it is only necessary to examine one set alone. I shall take the one shown in the first two columns of Table III of his paper. The value of $dG°(\text{sub})$ of eq 1–3 is obtained by adding twice the values given for binding by the α and β chains ($\delta_{4\alpha}$ and $\delta_{4\beta}$ of the table), while the value of $dG°(\text{tet})$ results from addition of the four free energies of binding by the tetramer ($\Delta G_{41}'$ to $\Delta G_{44}'$ of the table). The results of these operations, expressed in kcal/mol, are as follows:

	$dG°(\text{sub})$	$dG°(\text{tet})$	difference
first order	−33.48	−27.31	−6.17
second order	−20.80	−27.30	+6.50

The first-order results ($|dG°(\text{sub})| > |dG°(\text{tet})|$) correspond

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to a molecule that *dissociates* upon oxygen binding, and the second-order results ($|\mathrm{d}G^\circ(\mathrm{sub})| < |\mathrm{d}G^\circ(\mathrm{tet})|$) to a molecule that *associates* upon oxygen binding. The same conclusion may be reached by noticing that the free energy changes in subunit interactions upon ligation that are necessary to recover the experimental values of the four free energies of oxygen binding ($\{\alpha\alpha\}$ and $\{\alpha^i\beta^j\}$ of the table) are uniformly negative quantities in the case of first-order couplings (they antagonize ligand binding) and positive quantities in the case of second-order couplings (they facilitate ligand binding). The four free energies of oxygen binding to hemoglobin can be reproduced equally well by negative or positive subunit interactions; the concept, if not the exact numerical computation, has been clear since the original treatment of the subject by Pauling in 1935. However, the more recent knowledge that in hemoglobin subunit interactions antagonize oxygen binding, and reciprocally oxygen binding decreases the affinities of the subunits for each other, excludes positive changes of free energies of subunit interaction upon oxygen binding from consideration. In consequence, the original conclusion of Weber (1984) still stands: The free energy couplings in hemoglobin are, unequivocally, of first order.

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CORRECTIONS

Transfer of Long-Chain Fluorescent Free Fatty Acids between Unilamellar Vesicles, by Judith Storch and Alan M. Kleinfeld*, Volume 25, Number 7, April 8, 1986, pages 1717–1726.

Page 1722. Equations 5 and 7 should read

$$\mathrm{d}C_2(t)/\mathrm{d}t = k_{12}C_1(t) + k_{\mathrm{on}}[\mathrm{D}]C_3(t) - k_{21}C_2(t) - k_{\mathrm{off}}C_2(t) \tag{5}$$

$$\mathrm{d}C_4(t)/\mathrm{d}t = k_{12}C_5(t) + k_{\mathrm{on}}[\mathrm{A}]C_3(t) - k_{21}C_4(t) - k_{\mathrm{off}}C_4(t) \tag{7}$$

Please note that the solutions described in the paper were obtained with the correct equations.

Bromoacetophenone as an Affinity Reagent for Human Liver Aldehyde Dehydrogenase, by Alexander D. MacKerell, Jr., Robert S. MacWright, and Regina Pietruszko*, Volume 25, Number 18, September 9, 1986, pages 5182–5189.

Page 5187. Due to a publishing error, in column 2 two sentences on lines 48–55 have been duplicated and should be deleted.