Analysis of the Order of Free Energy Couplings between Ligand Binding and Subunit Assembly in Human Hemoglobin†

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ABSTRACT: The concept of free energy couplings has been extensively used in studies of the ligand-linked subunit assembly of oligomeric proteins such as human hemoglobin A [cf. Ackers, G. K. (1980) *Biophys. J. 32*, 331–346]. Recently, the concept of "order" of free energy couplings has been introduced as a description of the number of protein subunits that must be liganded to effect changes in intersubunit interactions [Weber, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7098–7102]. That report utilized the concept of order of free energy couplings to analyze a set of previously published equilibrium constants derived from data pertaining to the chemical equilibrium between oxygen and stripped hemoglobin A [Mills, F. C., Johnson, M. L., & Ackers, G. K. (1976) *Biochemistry 15*, 5350–5362]. The Weber report claims to have "unequivocally" demonstrated that the coupling between oxygenation and subunit assembly in hemoglobin A is "first order". In the present report, it is demonstrated that free energy couplings of both the first and second order are capable of describing the original oxygen binding data.