

Oxygenation-Linked Subunit Interactions in Human Hemoglobin: Experimental Studies on the Concentration Dependence of Oxygenation Curves[†]

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ABSTRACT: An experimental study on the concentration dependence of oxygenation curves for human hemoglobin has been carried out between 4×10^{-8} M heme and 5×10^{-4} M heme in 0.1 M tris(hydroxymethyl)aminomethane hydrochloride, 0.1 M NaCl, 1 mM disodium ethylenediaminetetraacetic acid, pH 7.4, 21.5 °C. With decreasing hemoglobin concentration the curves show pronounced shifts in position and shape, consistent with dissociation of tetrameric hemoglobin into dimeric species of high affinity and low cooperativity. Combination of these data with independently determined values of dissociation constants for unliganded and fully liganded hemoglobin permits a resolution of the seven parameters necessary to define the linked binding and subunit association processes. The total oxygenation-linked subunit dissociation energy (6.34 kcal) was resolved into intersubunit contact energy changes between $\alpha\beta$ dimers in tetrameric hemoglobin which accompany binding of the first, middle two,

and last oxygen molecules. The resolution is accurate to within approximately ± 0.3 kcal. To within this limit the isolated dimers are found to bind oxygen noncooperatively and with the same affinity as isolated α and β chains. Equally good fits to the data are obtained when dimers are slightly anticooperative. At least three major energetic states are apparently assumed by hemoglobin tetramers, with respect to the $\alpha^1\beta^2$ contact region, corresponding to (a) unliganded, (b) singly liganded, (c) triply and quadruply liganded species. The results do not establish whether these states may be assumed by a single molecule, or whether they arise as averages over a distribution of conformational states. They do provide unequivocal evidence against a concerted transition at any particular binding step in a system with only two energetic states of tetramer (i.e., an all or none switchover between T and R states at a particular binding step).