Oxygenation-Linked Subunit Interactions in Human Hemoglobin: Analysis of Linkage Functions for Constituent Energy Terms†

Michael L. Johnson,‡ Herbert R. Halvorson,§ and Gary K. Ackers*

ABSTRACT: Resolution of the linkage functions between oxygenation and subunit association–dissociation equilibria in human hemoglobin into the constituent microscopic terms has been explored by numerical simulation and least-squares analysis. The correlation properties between parameters has been studied using several choices of parameter sets in order to optimize resolution. It is found that, with currently available levels of experimental precision and ranges of variables, neither linkage function can provide sufficient resolution of all the desired energy terms. The most difficult quantities to resolve always include the dimer–tetramer association constant for unliganded hemoglobin and the oxygen binding constants to $\alpha\beta$ dimers. A feasible experimental strategy for overcoming these difficulties lies in independent determination of the dimer–tetramer association constants for unliganded and fully oxygenated hemoglobin. These constants, in combination with the median ligand concentration, provide an estimate of the energy for total oxygenation of tetramers which is essentially independent of the other constituent energies. It is shown that if these separately determinable parameters are fixed, the remaining terms may be estimated to good accuracy using data which represents either linkage function. In general it is desirable to combine information from both types of experimental quantities. A previous paper (Mills, F. C., Johnson, M. L., and Ackers, G. K. (1976), *Biochemistry, 15,* the preceding paper in this issue) describes the experimental implementation of this strategy.