

The Linkage between Oxygenation and Subunit Dissociation in Human Hemoglobin. Consequences for the Analysis of Oxygenation Curves[†]

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ABSTRACT: For human hemoglobin, a pronounced dependence of oxygenation curves upon protein concentration can be demonstrated experimentally in the range between 10^{-4} and 2×10^{-6} M heme. The effects of such protein concentration dependence upon analysis of saturation curves have been explored using a model-independent linkage analysis which incorporates the dissociation of tetramers to dimers. We have carried out simulations of oxygenation curves representing a variety of energy distributions designed to cover a wide range of values which are relevant to known hemoglobin systems and experimental conditions. The resulting simulated oxygenation curves were analyzed by least-squares minimization procedures in terms of the tetramer binding isotherm to yield the four apparent Adair con-

stants. These derived constants were compared with the originally assumed values used in the simulation in order to assess the extent to which their values may be altered by the presence of dimer. For each energy distribution the analysis has been carried out over a wide range of protein concentration. We have found that the presence of even small amounts of dimer that are necessarily present at the low protein concentrations commonly employed may have a devastating effect upon the reliability of Adair constant determinations. In addition to these simulated cases, we have analyzed two sets of highly precise experimental data from the literature in order to assess the degree to which constants obtained may have been influenced by the presence of dimer.