Kinetics of Deoxyhemoglobin Subunit Dissociation Determined by Haptoglobin Binding: Estimation of the Equilibrium Constant from Forward and Reverse Rates†

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ABSTRACT: Deoxyhemoglobin tetramers dissociate into dimers very slowly, with half-times on the order of several hours. It is demonstrated that absorbance changes in the Soret region which accompany this dissociation and persist upon binding of haptoglobin 1-1 to the dissociated dimers can be used for accurate kinetic determinations over the necessarily long periods required for study. This method of study for the slow reactions depends upon long-term spectral integrity of the reaction mixtures and upon accurate measurement. The variation in rate constants determined by this procedure has been correlated with variations in structural constraints at the dimer–dimer contact region. In the presence of 2,3-diphosphoglycerate the rate constant is decreased, consistent with the role of this effector in binding to both β chains and stabilizing the constrained deoxy tetramer against dissociation into $\alpha\beta$ dimers. With hemoglobin specifically modified (des-Arg-141α) to eliminate half the constraining salt links within the dimer–dimer contact region, the dissociation rate is increased by approximately three orders of magnitude. In hemoglobin S where the amino acid substitution is not directly in the intersubunit contact region of interest, the dissociation rate is found to be approximately the same as that for hemoglobin A. Combination of the dissociation rate constants determined by haptoglobin binding with stopped-flow determinations of the rate constant for reassociation of dissociated dimers provides an estimate of the equilibrium constant, ${}^0K_2$, for the deoxyhemoglobin dimer–tetramer equilibrium. This estimate is independent of any assumptions regarding other energetic quantities, and yields a value of $2.54 \pm 0.7 \times 10^{10}$ M^{-1} (heme) in 0.1 M Tris-HCl, 0.1 M NaCl, and 1 mM EDTA, pH 7.4, 21.5 °C. Thus the intersubunit contact energy is -14.0 ± 0.2 kcal/mol of heme. The stabilization energy between deoxy and oxy tetramers is found to be approximately 6.4 kcal/mol, under these conditions.