THE KINETICS AND MECHANISM OF THE REACTION BETWEEN SERUM ALBUMIN AND AURANOFIN (AND ITS ISOPROPYL ANALOGUE) *IN VITRO*

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The first detailed kinetic study of in vitro reactions between serum albumin and the second-generation gold drug Auranofin [$Et_3PAuSATg$ = triethylphosphine-(2,3,4,6-tetra-O-acetyl-1-ß-D-glucopytanosato-S-) gold(I)] and its tri-i-propylphosphine analogue, $iPr_3PAuSATg$, are reported. The reactions were investigated using Penefsky spun columns and NMR saturation transfer kinetics. Based on the Penefsky column data, the binding of the $Et_3PAuSATg$ to AlbSH (0.600 mM) was complete when gold concentrations were limiting: 0.093, 0.151, and 0.225 mM. The reaction is biphasic. The fast phase is defined by a first order rate constant, (k_1 = 2.94 ± 0.24 x 10^{-2} sec^{-1}) and accounts for ≈ 95% of the Au(I) bound. This phase is first order with respect to albumin, and zero order with respect to auranofin. A minor, slower step (k_2 = 2.26 ± 0.26 x 10^{-3} sec^{-1}), which accounts for only 5% of the reaction, is also first order with respect to albumin, and zero order with respect to auranofin. For $iPr_3PAuSATg$, only the first step was observed, k_1 = 1.4 ± 0.1 x 10^{-2} s^{-1}, and is first order in albumin and independent of $iPr_3PAuSATg$ concentration. ^{31}P-NMR saturation transfer experiments utilizing the auranofin analogue, $iPr_3PAuSATg$, under equilibrium conditions with excess $iPr_3PAuSATg$ and ATgSH yielded second order rate constants for both the forward (1.0 x 10^2 M^{-1} sec^{-1}) and the reverse (3.9 x 10^1 M^{-1} sec^{-1}) directions. A multi-step mechanism involving a conformationally altered albumin species to which gold binds was developed using the steady-state approximation. The mechanism accounts for the different reaction orders observed under the two set of conditions. A rate determining conformational change on the albumin governs the reaction as monitored by the Penefsky columns. A second order reaction of $R_3PAuSATg$ at Cys-34 is observed under the NMR conditions. These results are the first quantitative determination of auranofin-albumin reaction kinetics and the first mechanistic study of the reaction.

A novel binding mechanism, association of auranofin in the pocket of albumin-disulfide species can be detected by Penefsky and Hummel-Dreyer gel chromatographic techniques, but not by conventional gel-exclusion chromatography. This complex albumin-auranofin complex is weakly-bound and readily dissociates.