It has been proposed that annexin I has two separate interaction sites that are involved in membrane binding and aggregation, respectively. To better understand the mechanism of annexin-I mediated membrane aggregation, we investigated the properties of the inducible secondary interaction site implicated in membrane aggregation. Phospholipid monolayers, containing POPC/POPE/POPS (2:5:2) were formed on an aqueous subphase buffer (10 mM HEPES-KOH, 0.1M KCl, pH 7.0) and compressed to a surface pressure of 34 dynes/cm. Annexin I (or V) was injected into the subphase. X-ray specular reflectivity measurements showed that the thickness of annexin I layer bound to the phospholipid monolayer was 31±2Å, indicating that annexin I binds membranes as a protein monomer of monolayer in the presence of 1 mM Ca²⁺. Binding did not occur in the absence of Ca²⁺. Similar results were shown for annexin V. These features for annexin-I can be seen clearly in the figure below. When combined with surface plasmon resonance measurements, these results support a hypothetical model of annexin-I mediated membrane aggregation, in which a laterally aggregated monolayer of membrane-bound annexin I directly interacts with a secondary membrane via its induced hydrophobic interaction site (to be published in Biochemistry).