Screening; Purification; and Identification of a Copper-dependent FITC-binding Protein in Human Plasma: Albumin

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Abstract

In this study, a protein purified by fluorescein isothiocyanate (FITC)-affinity chromatography from human plasma was identified as albumin by MALDI-TOF-MS. Albumin was found to conjugate with FITC-labeled molecules through a copper-dependent reaction. The formation of this complex was confirmed by methods including a newly developed "charcoal-based fluorescence assay" (CFA), gel-filtration, affinity chromatography, and ultrafiltration. The binding was identified as disulfide bridge formation. This is the first to demonstrate that copper induces a covalent binding of FITC-labeled molecules with albumin. In addition, the developed CFA method facilitates the screening of small fluorescent dyes binding to macromolecules.